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Understanding the fate of active pharmaceutical compounds in waters receiving poorly or untreated wastewater for the development of a dedicated environmental risk assessment approach

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Understanding the fate of active pharmaceutical compounds in waters receiving poorly or untreated wastewater for the development of a dedicated environmental risk assessment approach

by

Simone Bagnis

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Author's declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

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Publications

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Understanding the fate of active pharmaceutical compounds in waters receiving poorly or untreated sewage effluent for the development of a dedicated environmental risk assessment approach – Simone Bagnis

The global increasing consumption and production of active pharmaceutical ingredients (APIs) is of a growing environmental concern. The inherently biological activity of APIs creates a concern of potential ecotoxicological effects even at very low concentrations. Such risk is particularly exacerbated in low and low-middle income countries (LLMICs) where it is common the practice of direct discharge of untreated wastewater (DDUW) into surface waters, which creates a heavily polluted area downstream, described as the "impact zone". Little is known about the environmental fate of APIs in this area. Nevertheless, measured environmental concentrations (MECs) of LLMICs show higher concentrations than for high-income countries. Globally, the MECs for APIs in the "impact zone" are often above 0.01 $\mu g\ L^{-1}$, which would be sufficient to trigger the environmental risk assessment (ERA) refinement phase. Environmental concentrations are calculated with a conservative default dilution factor (DF) of 10, but in many countries observations show a lower value. Additionally, the traditional ERA considers sorption on the wastewater sorbents in the wastewater treatment plant as removal process. This does not happen in the case of the DDUW, where instead the sorbent are released and diluted into the receiving waters. This peculiar widely spread environmental scenario translates in the necessity of a dedicated environmental risk assessment impact zone inclusive approach, and supports the generation of scientific robust data about the environmental behaviour of APIs along and beyond its boundaries. Such a situation is therefore giving rise to scientific questions such as the effects of dilution of the untreated wastewater on the distribution of APIs between water and sorbents, i.e. sorption and desorption; the effects of the dilution on the biodegradation of such molecules in the receiving compartment, e.i. the compound half-life; and the development of a scientific approach to model the boundaries of the impact zone and to predict the environmental concentrations within and beyond.

With the aim of creating a framework for the generation of sound scientific information regarding such area a methodological approach was designed. A first phase of such methodology included laboratory studies about the most prominent occurring environmental fate processes, such as distribution and biodegradation. This information was used to propose a model for the determination of concentrations along and beyond the impact zone. In a subsequent phase it was conducted a fieldwork to both validate the first phase and to provide a real case for the modelling of environmental concentrations, and the inclusion of such methodology parallel to the traditional ERA.

A first study was conducted to investigate the effect of DFs <10 on the distribution of APIs between wastewater sorbents and receiving waters. The sorption was consistent with the APIs chemical properties. Dilution increased desorption of the basic and neutral APIs and correlated with their lipophilicity (R^2 >0.98). The data showed a clear trend in the desorption process of APIs that may lead to higher exposure risk than anticipated.

A second study aimed at investigating low levels of dilution on the biodegradation of APIs. Also, the extent of the impact zone was modelled using the biochemical oxygen demand (BOD) as proxy. The degradation half-lives of acebutolol and diclofenac increased with increasing dilution and resulted in higher environmental persistence. The other APIs investigated were not degraded, but acetaminophen which was quickly lost from the solution (<24 h). The temporal end boundary of the impact zone was predicted as 24 h. Therefore, it was concluded that most of the investigated compounds would persist beyond the impact zone, as defined by BOD concentrations owing to the fact that they exhibited greater persistence than the DOM comprising the BOD within the samples.

Thirdly, a sampling campaign of the Nairobi/Athi river catchment was performed to characterize the impact zone and the occurrence of APIs. The study showed a clear relationship with the DOM quality variation and the diffuse source areas of untreated wastewater within the city, as well as the occurrence of API trends. The study showed a first attempt of understanding the role of such an impact zone with respect to API source, occurrence and fate and furnishes pivotal information to further investigate such areas.

In conclusion, from the results obtained by the laboratory studies and fieldwork it clearly results on the impact zone as an area of severe pollution which needs a dedicated ERA approach. Beyond its boundary the classical protocol of ERA can be applied, but to estimate the predicted environmental concentrations of APIs it is necessary to create a model to be applied to such area. Such model would output environmental concentrations beyond the end boundary of the impact zone that may be used for further risk assessment. Further studies are necessary to provide scientific robust endpoints useful to the development of such model.

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List of most frequent abbreviations

APIs	Active pharmaceutical ingredients
ACE/ACB	Acebutolol
AMI	Amitriptyline
BOD	Biological oxygen demand
CEC	Cation exchange capacity
COD	Chemical oxygen demand
DCF	Diclofenac
DDUW	Direct discharge of untreated wastewater
DF	Dilution factor
DOM	Dissolved organic matter
EE2	17-α ethynylestradiol
EEM	Excitation-emission matrix
EMA	European medicine agency
ERA	Environmental risk assessment
K	Partition coefficient

Koc	Organic carbon-water distribution coefficient
Kow	Octanol-water distribution coefficient
LLMICs	Low and low-middle income countries
MECs	Measured environmental concentrations
OECD	Organization for economic co-operation and development
OFX	Ofloxacin
PBT	Persistent, bioaccumulating and toxic
PEC	Predicted environmental concentrations
PIE	Pharmaceuticals in the environment
PKa	Acid dissociation constant
PNEC	Predicted no effect concentrations
POPs	Persistent organic pollutants
SS	Suspended solids
SW	Synthetic wastewater
TDS	Total dissolved solids

TOC	Total organic carbon
TS	Total solids
TSS	Total suspended solids
VLS	Valsartan
VOCs	Volatile organic compounds
WWTW	Wastewater treatment work

1. WASTEWATER AND PHARMACEUTICALS IN THE ENVIRONMENT

Overview

The increasing consumption and production of active pharmaceutical ingredients (APIs) in low and low-middle income countries (LLMICs) is of a growing environmental concern owing to potential ecotoxicological effects and antibiotic resistance. This is related to the practice of direct discharge of untreated wastewater (DDUW) into receiving waters, which creates a heavily polluted area downstream, named the "impact zone". Little is known about the environmental fate of APIs in this area. Nevertheless, a few available measured environmental concentrations (MECs) in LLMICs show higher concentrations than for high-income countries with developed wastewater treatment infrastructures. Globally, the MECs of APIs in the "impact zone" are often above 0.01 μg L^{-1}, which would be sufficient to trigger the environmental fate refinement of the European Medicine Agency (EMA) environmental risk assessment (ERA). In the ERA predicted environmental concentration (PEC) calculation, a dilution factor (DF) of 10 is used as a default, but in at least 53 countries worldwide, the local predicted DF median value is less than this. There is no information available in the literature about the effects of low dilutions on the natural attenuation and resulting exposure of APIs, and especially in the specific case of DDUW. This study provides information about effects of the dilution of untreated wastewater on the partitioning – sorption and desorption – and biodegradation of APIs within the impact zone. Also, a sampling campaign for a real environmental catchment allowed an investigation into an actually field-based impact zone. This research provides pivotal information which will support the development of an ERA approach to the impact zone and beyond; based on novel, science-led principles.

1.1. Introduction

1.1.1. Pharmaceuticals in the environment

Modern society largely depends on the use of chemicals that have the potential for adverse environmental effects. Public concern began with the publication of Rachel Carson's "Silent spring" in 1962, which drew attention to the effects of pesticides on the environment and the consequent risks to humans. Although persistent organic pollutants, "POPs", which are persistent, bioaccumulating and toxic (PBT) chemicals, have largely been regulated via global agreements such as the Stockholm Convention held in 2001 (http://chm.pops.int/Home/tabid/2121/Default.aspx, 2017), new compounds are continually being created and employed in many applications. Some of these new substances are collectively classified under the name of "emerging contaminants", which are defined as synthetic or natural chemicals detected and monitored in the environment and thought to pose a potential risk of harmful effects to human health or ecosystems (Sorensen et al., 2015).

Due to improvements in analytical technology, many new compounds can be detected in environmental matrices at very low concentrations (< 1 ng L^{-1}). Active pharmaceutical ingredients (APIs) have received attention, particularly since the studies by Purdom et al. (1994) and Sumpter and Jobling (1995) reported that nanograms per liter concentrations of 17α-ethinylestradiol (EE2) could cause feminization of river fish. Consequently, the perception of potential detrimental effects of APIs on the environment led to the creation of a dedicated area of study, named "Pharmaceuticals in the Environment" (PIE) (Daughton and Ternes, 1999). More recently, the near extinction of several vulture species in India caused by diclofenac toxicity provided further evidence of negative environmental impacts of some APIs (Swan et al., 2006). These findings

raised questions about the possibility of other APIs having similar disruptive effects in the environment (Fatta-Kassinos et al., 2011). The increased number of studies demonstrating API occurrence in surface and groundwater worldwide and evidence of ecotoxicological effects have further increased concern (Nikolaou et al. 2007; Brausch et al. 2012; Arnold et al. 2014; Backhaus 2014; Brodin et al. 2014; Brown et al. 2014; Gaw et al. 2014; Hutchinson et al. 2014; Kookona et al. 2014; Küster and Adler 2014; Lalone et al. 2014; Larsson 2014; Shore et al. 2014). In addition, the global pharmaceuticals market is expanding year on year and was valued at $1.072 trillion in 2015, and a significant number of APIs have been detected in sewage effluents leading to concern about the ecosystem impact of these emerging contaminants (Comber et al., 2018; Gardner et al., 2013, 2012).

APIs comprise a large and diverse group of compounds used for the prevention, cure or treatment of diseases in humans and livestock. Currently, there are uncertainties associated with the environmental risk assessment of pharmaceuticals due to lack of knowledge concerning their fate in wastes and the environment, their uptake, metabolism and excretion (pharmacokinetics) in wildlife, and their target affinity and functional effects (pharmacodynamics) on non-target species (Arnold et al., 2014). The environmental threat is linked to some structural features, which may result in *inter-alia*, persistence to degradation, high bioavailability and high solubility. Furthermore, APIs are excreted after ingestion either as parent compounds or metabolic products, which can be, naturally or by wastewater treatment processes, converted to transformation products or back to the parent compound creating further uncertainty regarding environmental fate and ecotoxicological effects (Boxall et al., 2012). In fact, the metabolism of xenobiotic compounds in organisms plays a key role in reducing drugs toxicity, and fosters their

excretion through bile, faeces and urine. The main metabolic goal is to transform lipophilic compounds to more hydrophilic ones that can thus be more easily excreted (Hutchinson et al., 2014). API's and xenobiotics are converted into metabolites by drug metabolizing enzymes, or excreted from cellular environments. The drug can be excreted as parent compound (up to 50-90% of the dose), which means it is chemically unchanged and in its pharmacologically active form; examples of this include amoxicillin and ciprofloxacin (Githinji et al., 2011). The trend in general, however, is that the metabolites in excretions are less bioactive than their parent compound form (Daughton and Ternes, 1999). However, it is also necessary to consider the evidence that conjugates can be hydrolysed by bacteriological activity in sewerage works or in the environment, and retransformed to their parent compound form (Hirsch et al., 1999).

1.1.2. Sources and fate of pharmaceuticals in the environment

The introduction of APIs in the environment occurs chiefly from point sources and, despite some of them being highly degradable, continuous inputs can result in pseudo-persistent behavior (Grenni et al. 2013). The principal source of APIs away from rural areas is via human excretion (private household and hospitals), and the consequent discharge of treated and untreated wastewater into the aquatic environment (Daughton and Ternes, 1999; Kookana et al., 2014; Malik et al., 2015). Industrial manufacturing plants are considered important point sources of pharmaceuticals to the environment (Cardoso et al. 2014; Larsson et al. 2015), as well as livestock farming and aquaculture in rural areas, which are regarded as additional significant sources of veterinary pharmaceuticals (Carmosini and Lee, 2009; Shimizu et al., 2013; Song and Guo, 2014). Also, improper disposal of expired medicinal products contributes to the load, although is considered a minor impact (Tong et al., 2011).

depicts the pathways of pharmaceuticals to the environment as well as how they may find their way back into the food chain (Benotti et al., 2009; Heberer, 2002).

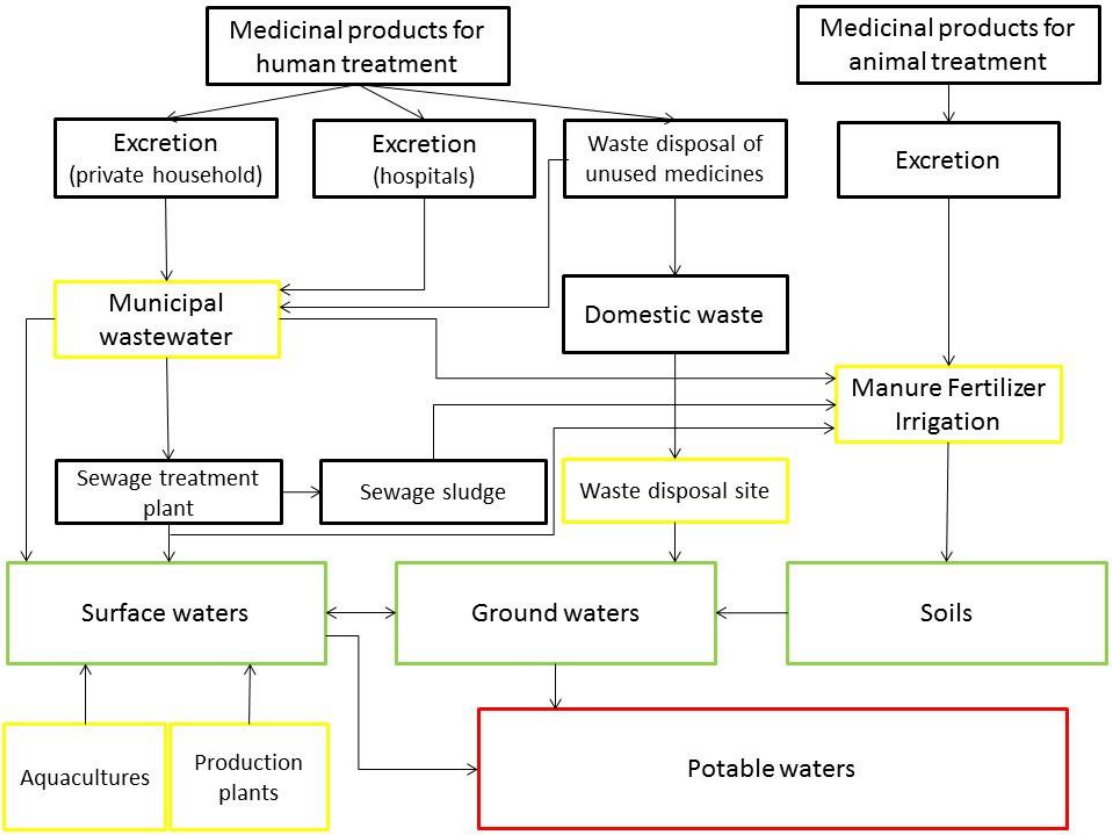


Figure 1 Sources and routes of pharmaceuticals to the environment and the intermediate steps encountered between the point source and the receiving environmental compartment or drinking water for human consumption (Heberer, 2002). Yellow boxes depict the main sources to the environment; green boxes depict the receiving environmental compartments; the red box depicts potable water, a direct source of human exposure (Bagnis et al., 2018b).

1.1.3. Wastewater and the environment

1.1.3.1. Wastewater

The production of waste by human activities is unavoidable, and a proportion ends up as wastewater. The management of wastewater by urban administrators has a long history. Many relicts of ancient urban wastewater management systems have been found in early cities in Eurasia, from sewer and storm water drainage systems dating back to the Minoan civilization, around the second millennium BC, to the fundamental basis of the modern sewerage systems laid in the Ancient Greece (Angelakis et al., 2005). The Greek sewerage systems can be compared only to the modern ones engineered during the 19th century, which fascinated Victor Hugo to such an extent to write in *Les miserables* that "*The history of men is reflected in the history of sewers*" and "*it has been a sepulcher, it has served as asylum, crime, cleverness, social protest, the liberty of conscience, thought, theft, all that the human law persecutes or have persecuted is hidden in that hole*" (Lofrano and Brown, 2010). Wastewater is, both romantically or scientifically, a source for sociological analysis (Lofrano and Brown, 2010), as, for instance, when used to determine the use of drugs in a community (legal or otherwise) through the measurement of sewage drug biomarkers (Castiglioni et al., 2013). Again from an historical perspective, it is interesting to mention the timeline of the sanitation evolution drawn by Lofrano and Brown (2010), which highlights five distinct historical periods based on the level of sanitation development: early history as the period antecedent the Roman period, ending in 465AD with the beginning of the so-called "dark age of sanitation", lasting from the middle ages to the industrial revolution. In the most recent days the traditional problems associated with wastewater have been solved by developed countries, whilst in those countries characterized by a middle and middle-low

income the problem is still concerning because of the lack of access to sanitation of a large fraction of the population (Kookana et al., 2014). An instance are the “flying toilets” of Kibera, a degraded area in Nairobi, where owing to the lack of sanitation people throw excreta in the streets, similar to the middle ages (Lofrano and Brown, 2010).

The main types of wastewater are domestic wastewater, principally composed of black-water (excreta, urine and faecal sludge) and grey-water (bathing and kitchen wastewater); industrial wastewater, discharged from manufacturing facilities; wastewater from institutions like hospitals; and also infiltration into sewers, storm water, leachate, and septic tank wastewater. The composition and quality of the wastewater is largely variable between different human societies and industries. Such variability is determined by many factors such as lifestyle, behaviour and standard of living of the inhabitants, as well as the juridical framework in use in a specific region (Henze and Comeau, 2008). For such a reason it is not possible to ‘standardise’ wastewater composition, even though it is possible to list the main components and to classify the different wastewaters based on the source (Tchobanoglous et al., 2003).

The wastewater constituents are mainly biodegradable organic materials, microorganisms, other organic chemicals (e. g. detergents, pesticides, oil and grease, pharmaceuticals, etc.), nutrients, metals, and other inorganic materials. Also some physical characteristics are typical of wastewaters such as high temperature waters, odour, and radioactivity. Organic matter is the main pollutant carried by wastewaters, and it is measured as biochemical oxygen demand (BOD) which if not reduced in concentration via treatment, acts as an energy source to surface water microbial communities whose growth leads to the stripping the oxygen out of the system ending in anoxia and death of invertebrates and fish.. The other components contribute to the

toxicity of the wastewater as well, and are of importance during the biological treatment in wastewater treatment works (WWTW) and in the receiving surface waters.

1.1.3.2. Wastewater treatment

Once generated the wastewater is collected by sewerage systems and conveyed to a WWTW where it undergoes various treatment processes aimed at reducing the pollutants concentration, namely primary treatment, secondary treatment, and in some cases, tertiary and quaternary treatments, the latter two also defined as advanced treatments.

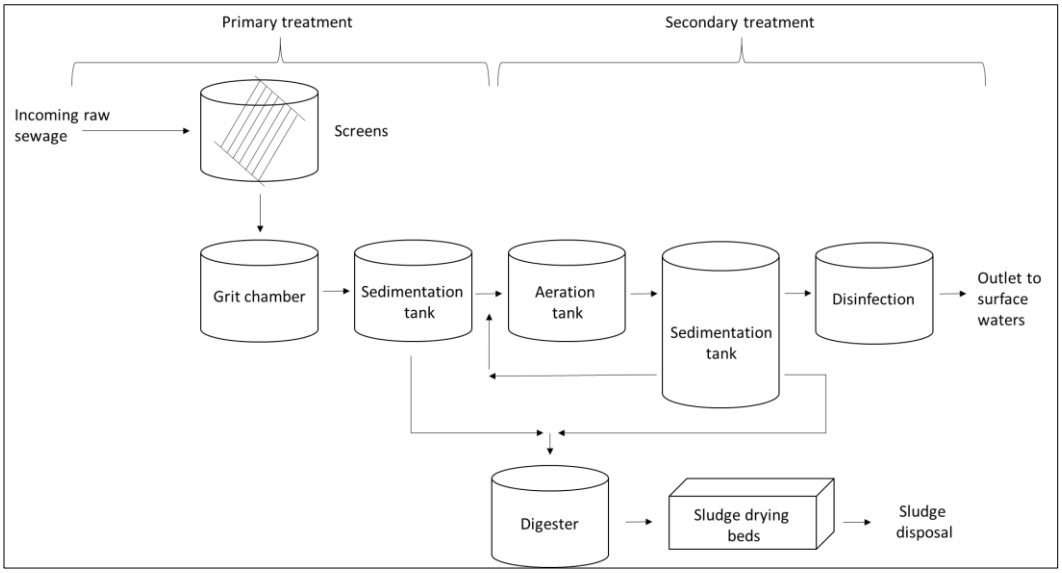


Figure 2 Diagram describing the basic primary and secondary treatment processes in a wastewater treatment plant.

The primary treatment mainly consists in the removal of the heavier settleable material by gravitation in sedimentation tanks (Figure 2). This is the first kind of wastewater treatment in history, some forms were also found in ancient cities, and it is the most common form of wastewater treatment (Lofrano and Brown, 2010). More recently (since about 1900), biological treatment, described as 'secondary treatment',

was developed subsequent to primary settlement, which involves the use of micro-organisms for the conversion of the organic material to carbon dioxide, water and energy for growth (Figure 2). The secondary treatment can be of two basic types: attached growth and suspended growth. The former consists of a population of microorganisms attached to a packing material. The wastewater flows through the space between the packing material, i.e. rocks, gravel, sand, redwood or plastic, and other synthetic materials, and the pollutants carried by the wastewater are removed by the attached growth, or also called biofilm. In the latter secondary treatment method, the microbial population is maintained in suspension by mixing the liquid in a properly aerated tank. Once the importance of the impact of wastewater on the environment and the return to human economics and health was realized some additional advanced treatment became more common in developed countries such as membrane systems, adsorption techniques, electrodialysis, and disinfection practices (Tchobanoglous et al., 2003).

1.1.3.3. Wastewater treatment global status

In many part of the world, and in particular in LLMICs in Asia, Africa and South America, wastewater receives only poor or partial treatment and is routinely discharged untreated in aqueous environments (Finnegan et al., 2009; Malik et al., 2015). The reason is often found in insufficient infrastructures owing to the rapid development of cities and the lack of funds for the necessary upgrade (Malik et al., 2015). This practice leads to an immediate serious ecological impairment caused by the main pollutants carried by the wastewaters, namely nutrients and other organic pollutants such as, for instance, pharmaceuticals (Corcoran et al., 2010). The excessive concentration of nutrients leads to algal blooms and eutrophication that itself leads to the consumption of all the oxygen available in the water with consequent negative impacts on the local biota, for instance

fish kills (Corcoran et al., 2010). The other organic pollutants are present at a lower concentration, but might be the cause bioaccumulation in the food chain up to top predators including humans. Of particular concern are those organics that can lead to endocrine disruption in organisms, which include APIs (Corcoran et al., 2010).

The wastewater treatment level varies with patterns recognisable at regional level. North America, Australia, and Europe show the highest performance in the wastewater treatment normalized per connection rate, followed by Middle East and North Africa, East Asia and the Pacific, Eastern Europe and Central Asia. The least performant regions are Latin America and Caribbean, Sub-Saharan Countries and South Asia (Malik et al., 2015). The performance is correlated to the income level of each region with a low income corresponding to low wastewater treatment and often greatest rate of population growth. These data are in accordance with estimates from the United Nations Environmental Programme (UNEP) which show that about the 80% of the wastewater produced in LLMICs is discharged directly into the environment (Malik et al., 2015).

A good example of continuous DDUW in fresh surface waters of LLMICs come from the Nairobi river basin in Kenya, which has recently shown evidence of consequent significant contamination by APIs (K'oreje et al., 2016, 2012; Ngumba et al., 2016). In Kenya, the national sewerage coverage is only 17% with variations between cities, for instance Nairobi (28%), Mombasa (4%), Kisumu (13%) (K' oreje et al., 2016; Malik et al., 2015). Additionally related Nairobi water quality, it should be noted that Nairobi is the fastest growing city in Sub-Saharan Africa and it is predicted to have a population growth from the actual 3.5 million to 7 million in the coming 20 years as a result of migration of people from the rural areas to the city (Mundia and Murayama, 2013); and this case is valid for most cities in Africa.

1.1.3.4. Impact zone concept

The DDUW lead to the formation of a severely polluted area downstream of the discharge point. Such an area was named “impact zone” for the first time at a workshop in 1995 (Limelette III workshop) (A.I.S.E./CESIO, 1995) and the concept has been further adopted by several authors (Finnegan et al., 2009; McAvoy et al., 2003; Whelan et al., 2007). The impact zone is defined as the area beginning at the discharge point source end ending when BOD and ammonia are quantified below their predicted no effect concentrations (Figure 3). Such a zone can be thought as a natural treatment system in which biodegradation is the main removal process. The factors determining the fate of the chemicals present in the impact zone are partitioning processes, which are responsible for environmental availability of the pharmaceutical compounds to the bacteria, and other toxicants that can be responsible for the decline of one or more bacterium species.

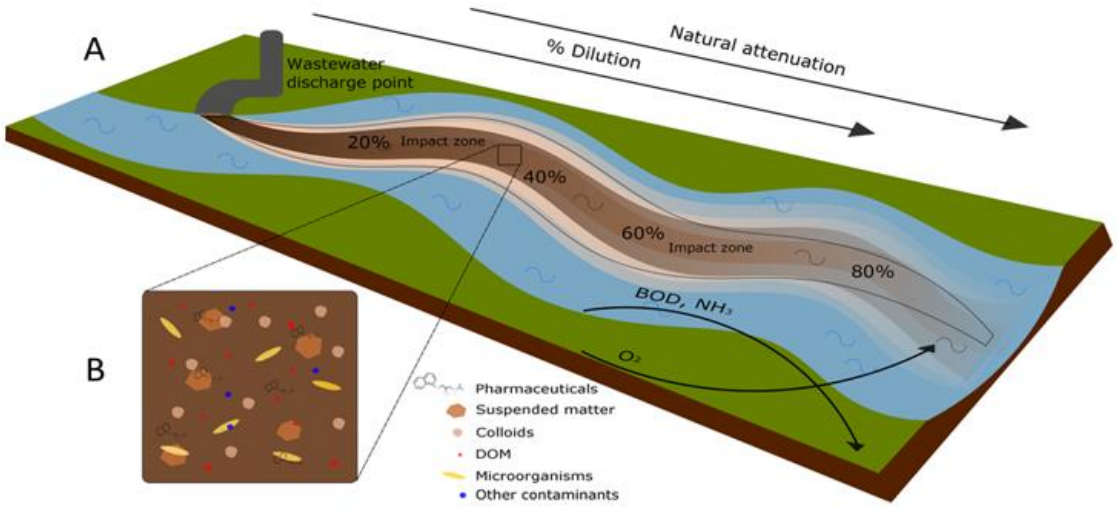


Figure 3 Representation of the impact zone; A: Impact zone, showing dilution and natural attenuation directions; B. Complexity of the untreated wastewater matrix (suspended matter, colloids, bacteria, DOM, APIs, other contaminants).

Little is known about the fate of APIs in this area, but investigations of continuous discharge of untreated wastewater in fresh surface waters come from the Nairobi/Athi catchment in Kenya, where it has been recently shown evidence of significant contamination by pharmaceuticals, detected in concentrations ranging from ng L^{-1} to 100 µg L^{-1} (K'oreje et al., 2016, 2012; Ngumba et al., 2016). Also, in Nigeria were detected concentrations of APIs at concentrations above 50 µg L^{-1} (Olatunde et al., 2014). Several studies showed high surface waters concentrations of APIs in South Africa up to 30 µg L^{-1} of atenolol (Agunbiade and Moodley, 2016, 2014), 85 µg L^{-1} of ibuprofen (Matongo et al., 2015), and 430 ng L^{-1} of several antiretroviral (Wood et al., 2015). Also, the wastewater of API factories was deemed as the main source of high concentrations, up to mg L^{-1}, in Pakistan (Ashfaq et al., 2017) and India (Larsson, 2014).

The background information in the section 1.1.4 is based on the paper published in Environmental Chemistry Letters available at https://doi.org/10.1007/s10311-018-0742-7.

1.1.4. Environmental natural attenuation processes

Once wastewater borne APIs enter into the environment they may undergo natural attenuation by physical, biological or chemical processes that reduce their concentration such as dilution (although sometimes excluded from such processes), transformation (photo-degradation; bio-degradation, hydrolysis) and sorption (Figure 4) (Gurr and Reinhard, 2006). Volatilization is also a common loss process, though APIs are considered negligibly volatile owing to low Henry's Law constants (Gurr and Reinhard, 2006). The importance of natural attenuation lies in the reduction of environmental concentration, and therefore potential toxicity of chemicals. The degree of such processes determines the fate of contaminants in the environment, namely their occurrence and distribution (Lin et al., 2010).

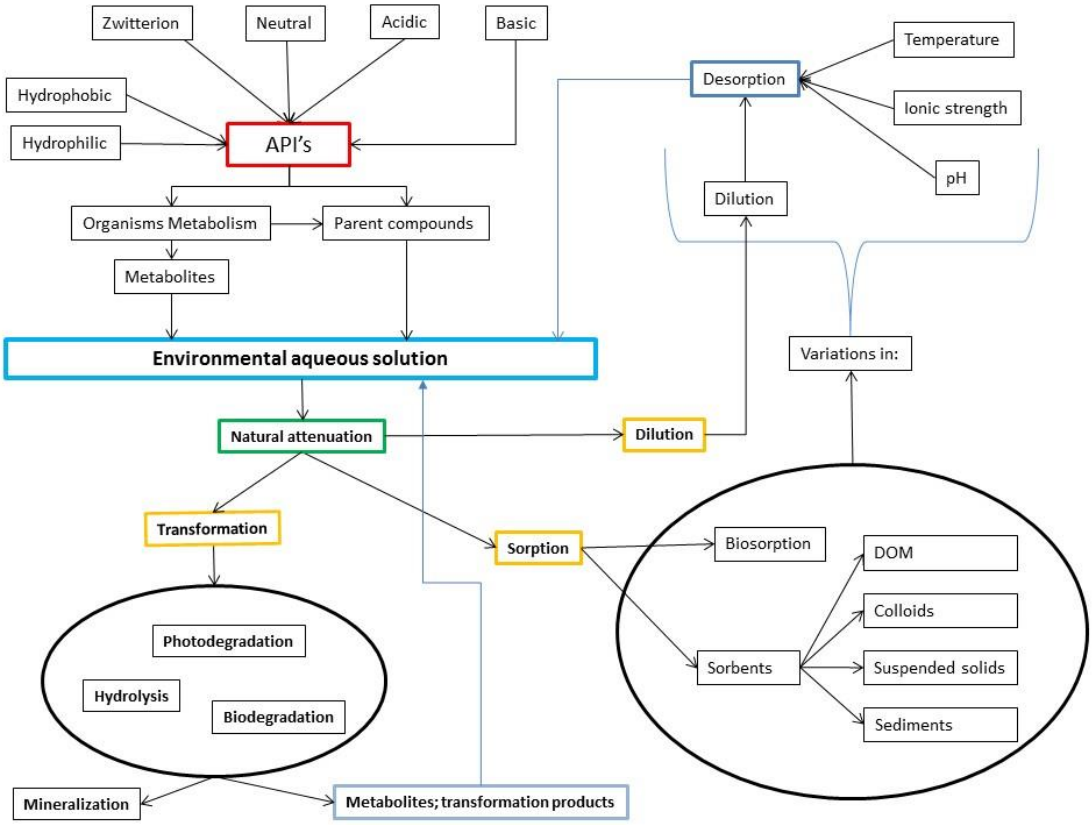


Figure 4 Fate of active pharmaceutical ingredients (APIs) in the environment and possible routes of return of parent compounds or metabolites caused by desorption or transformation processes; The red box refers to the APIs and possible chemical features (i.e. Polarity: hydrophobic or hydrophilic; Functionality: acidic, neutral, basic, zwitterion); The blue box depicts the environmental receiving compartment; the green box depicts the natural attenuation; the yellow boxes serve as subdivision of the three main attenuation mechanisms; the pale blue coloured arrows depict the route and causes of return to the aqueous environmental solution; the pale blue box the desorption mechanism. DOM is dissolved organic matter (Bagnis et al., 2018b).

1.1.5. TGD approach to producing PEC_{SW} for environmental risk assessment

The European Commission Technical Guidance document on Risk Assessment (TGD) includes a detailed section on how to calculate PEC in surface water. The PECs are calculated at both regional and local levels, the former used as background, as assumed to be constant in the environment, and summed to the latter, which instead describes a small standardized environment. The local surface water PECs are the focus of this research, which aims at proposing an approach for the particular case of direct discharge of untreated wastewater, not discussed in the technical guidance.

According to the TGD, the local PECs in surface water are calculated with the main assumptions that complete mixing of the effluent in surface water is the representative exposure situation for the aquatic eco-system; and that for the first approach in the local assessments, volatilisation, degradation, and sedimentation are not relevant processes because of the short distance between the point of effluent discharge and the exposure location, and therefore ignored.

After going through the wastewater treatment plant processes, and after discharge, the dilution is considered the main natural attenuation process and the sorption is also considered to play a consider role on the decrease of the local concentration of a determined chemical.

The TGD is meant to be a guidance for the calculation of the environmental risk assessment of a variety of chemicals and environments, and as such furnishes a widely accepted overall approach.

However, the particular local environment described as the impact zone in this research work is peculiar and worth a dedicated approach. In fact, the dilution in this case could lead the redistribution process of desorption, which can lead to underestimation of the actual PEC in the surface waters at the discharge point. This perspective on the calculation of PECs for surface water challenges the TGD assumption that the dilution is by default a natural attenuation process and instead raises the question of the role of desorption, especially at low levels of dilutions.

1.1.6. Environmental risk assessment of pharmaceuticals

The application for marketing of a pharmaceutical for human use needs to be accompanied by an environmental risk assessment (ERA) (EMA, 2006). The eventual impact caused by the introduction of an API into the environment does not constitute a reason for refusal in any case, however precautions to limit such impact need to be considered.

The assessment of the potential risk to the environment caused by a pharmaceutical is a step-wise approach consisting of two phases: the first phase (Phase 1) is an estimate of the level of exposure to an API in the environment. Depending on whether a potential risk is estimated may trigger the next phase, which corresponds of two parts, Tier A and B, which are aimed at refining the data about environmental fate and effects.

In phase 1, the estimation is based on the API only, irrespective of its route of administration, form, metabolism, and excretion. Regardless of the outcome, the compounds with a log K_{ow} above 4.5 are screened for persistence, bioaccumulation, and toxicity according to the EU TGD (European Comission Joint Research Centre, 2003).

In this primarily phase predicted environmental concentrations (PEC) are calculated with the following assumptions: a fraction of the overall market penetration, the amount used per year is evenly distributed by time and geographic area, the wastewater treatment plant (WWTP) is the main source of APIs to the environment, no attenuation due to biodegradation or sorption occurs in the WWTP, and metabolic transformations are not considered.

The PEC are therefore obtained according to Equation 1:

Equation 1

$$PEC_{SURFACE\ WATER} = \frac{DOSE_{ai} * F_{PEN}}{WASTE_{inhab} * DILUTION}$$

Where PEC is the predicted environmental concentration in surface waters (outcome); $DOSE_{ai}$ is the maximum daily dose consumed per inhabitant; F_{pen} is the fraction of market penetration with a default value of 0.01; $WASTE_{inhab}$ is the amount of wastewater per inhabitant per day with a default value of 200; and DILUTION is the dilution factor with a default value of 10.

If the PEC value is below 0.01 µg L^{-1} and no other apparent environmental concerns are present, the API is considered safe and no further studies are necessary; if

the PEC is above such a value, phase 2 – the environmental fate and analyses needs to be performed.

Phase 2 consists of an assessment considering the ratio of the PEC (that consider relevant datasets from environmental testing) to ecotoxicological data estimating the predicted no effect concentrations (PEC/PNEC) (Tier A). If these data are not considered sufficient then further testing is required (Tier B). Greater detail is provided in the guideline on the environmental risk assessment of medicines for human consumption issued by the European Medicine Agency (EMA, 2006).

1.2. Aims of the project

In the following list are described the main aims of the research:

1. Assessment of the effect of low levels of dilution (<10) of the sewage effluent mixed with receiving water on the partitioning of APIs, namely sorption and desorption.
2. Assessment of the effect of low levels of dilution (<10) of the sewage effluent mixed with receiving water on the biodegradation rates of selected APIs.
3. Development of a model to determine the extent of the impact zone based on BOD consumption, predicted from DOC concentrations.
4. Case study aimed at the characterization of the extent of a real impact zone and the occurrence of APIs in a real environment.
5. Outlining implications for the development of an API ERA approach for the impact zone.

1.3. Scope

As a preliminary study, a series of laboratory tests were performed to evaluate the effects of low levels of dilution on the natural attenuation processes occurring in the impact zone. The results were then evaluated against data obtained from field work. The scope was a basic provision for data useful in a preliminary assessment of the occurrence of APIs within the impact zone and beyond, and for the development of a dedicated ERA approach to address this.

1.4. Outline

This thesis begins with an introduction to the APIs as emerging environmental contaminants, the source and fate, and brief description of the global wastewater treatment status and on the formation of the impact zone as a result of direct discharge of untreated wastewater.

The second chapter is dedicated to a literature review about the sorption of APIs in surface fresh water sorbents. Information available in the literature is screened and collated, and a critical analysis has been undertaken.

Subsequently, in the third chapter, the materials and methods are described. Such as the ingredients and methodology used to create the synthetic wastewater, the rationale for the selection of the APIs to be investigated, and the analytical methodologies.

In the fourth chapter are the results and discussion about sorption and desorption as an effect of dilution of untreated wastewater into freshwater. Some implications of these findings for ERA are also provided.

The fifth chapter is dedicated to the description of the effects of low levels of dilution of untreated wastewater on the biodegradation of APIs. Also, modelling of the temporal extent of the impact zone based on the correlation of TOC and BOD is attempted and the results discussed together with possible implications for ERA.

The sixth chapter provides and discusses the data obtained from fieldwork in the Nairobi and Athi Rivers Catchment, Kenya. The data about the occurrence of a set of

56 APIs along a transect of the catchment are discussed as well as the extent of the impact zone. Also in this case are furnished implications for ERA.

In the conclusions are suggestions for the implementation of an environmental risk assessment approach including the impact zone as well as outlining the need for further studies.

2. PROCESSES OF DISTRIBUTION OF ACTIVE PHARMACEUTICAL COMPOUNDS IN SURFACE FRESHWATER ENVIRONMENTS AND IMPLICATIONS FOR ENVIRONMENTAL RISK ASSESSMENT

This experimental chapter was published as: Bagnis, S., Fitzsimons, M., Snape, J., Tappin, A., Comber, S., (2018). Processes of distribution of pharmaceuticals in surface freshwaters : implications for risk assessment. Environ. Chem. Lett.; and it is available online at the following DOI address: https://doi.org/10.1007/s10311-018-0742-7.

Abstract

Once in the environment, APIs can undergo processes of natural attenuation, i.e. dilution, sorption, transformation, depending on the inherent physico-chemical properties of the compound, such as water solubility, lipophilicity, vapour pressure, and environmental conditions, such as pH, temperature and ionic strength. A key natural attenuation process is sorption onto surface water components, namely dissolved organic matter (DOM), colloids, suspended solids and sediments, which control APIs distribution, residence time and persistence in aquatic systems. Here we review studies of sorption capacity of such sorbents to APIs which report on the importance of several environmental and sorbent-specific properties, such as the composition, quality, and amount of the sorbent, and the environmental pH, which determines the speciation of both the sorbent and compound. The major points are the importance of processes of distribution on freshwater sorbents for the determination of environmental concentrations of pharmaceuticals; the reliability of surrogate standards for DOM distribution; the critical evaluation of the significance of the distribution processes on attenuation of APIs; and the implications of considering this information in the APIs environmental risk assessment protocol.

2.1. Introduction

Attenuation of APIs through partitioning onto sorbents in surface waters can be significant in controlling distribution, residence time and persistence in aquatic systems (Lützhøft et al., 2000); as a result ecotoxicological effects might be either reduced, as the pharmaceutical is less bioavailable, or increased through bioconcentration (Ra et al., 2008). The extent and reversibility of partitioning depends on both the pharmaceutical and sorbent surface characteristics (Delle Site, 2001). The degree of partitioning is expressed by the partition coefficient (K), which is the particulate:solution concentration ratio of the pharmaceutical. The pharmaceutical functionality, basic, acidic, neutral or zwitterion, is critical in controlling its fate. For ionisable compounds, where the degree of ionization is pH-dependent, expressed by the acid dissociation constant, pK_a. Figure 5 demonstrates the ionisation behaviour of molecules representing basic, acidic and zwitterion pharmaceuticals at pH 5 – 9. Basic compounds are fully ionised at the lower pH and the neutral fraction abundance increases above pH 7 (Figure 5A), whilst acidic pharmaceuticals demonstrate converse behaviour (Figure 5B). The zwitterion, ofloxacin, contains at least one charge over the pH range (Figure 5C). These pH-related changes in the abundance of each species affect their distribution in aquatic environments.

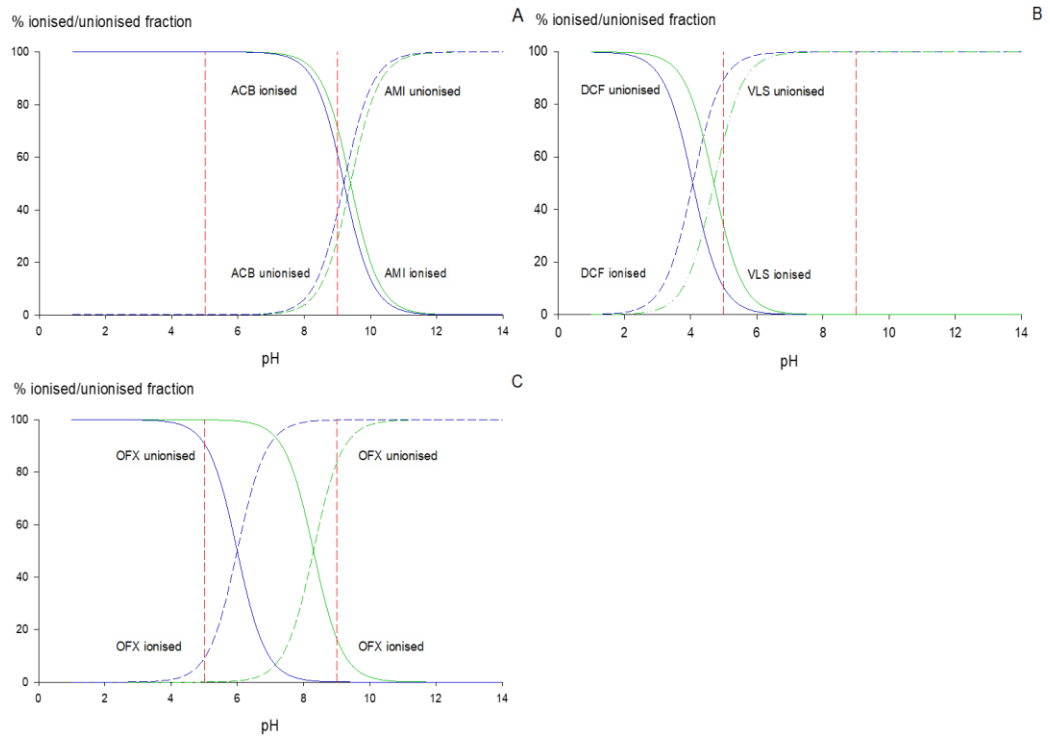


Figure 5 pH-dependent ionization for A. two basic compounds, amitriptyline (AMI) (pKa 9.4) and acetobutol (ACB) (pKa 9.2) B. two acidic compounds, diclofenac (DCF) (pKa 4.0) and valsartan (VLS) (pKa 4.7). C. the zwitterion ofloxacin (OFX) (pKa basic 8.3, acidic 6.0). All the functionalities show a unionised fraction environmental pH range (red lines).

The main sorbents in aquatic environments are dissolved organic matter (DOM), colloids, suspended solids and sediments.

2.1.1. Dissolved organic matter

DOM is a biogeochemical product which interacts with organic pollutants in water (Leenheer and Croué, 2003). It can be derived from natural or anthropogenic sources and is present in natural waters at concentrations ranging from 0.1 mg L^{-1} in groundwater to above 300 mg L^{-1} in wastewater (Leenheer and Croué, 2003; Tchobanoglous et al., 2003). DOM cannot be completely characterized but is regarded as a mixture of aliphatic and aromatic compounds with some predominant chemical functionality, including hydroxyl,

carboxyl, amido and keto groups (Leenheer and Croué, 2003). For experimental purposes, environmental DOM is physically filtrated from water, typically 0.45 or 0.2 µm membranes, and chemically fractionated (e.g. via sorption and elution from resins) in order to obtain operationally-defined DOM fractions, namely hydrophobic or hydrophilic, and the acidic, basic or neutral sub-classes (Filella 2009; Maoz and Chefetz 2010). Further classification is based on fluorescence excitation-emission matrix patterns, which show distinct "protein-like" and "humic-like" peaks (Hudson et al., 2007). DOM in aquatic environments can be derived from *in situ* autochthonous sources by bacterio-plankton activity, or introduced via carbon recycling (Michael-Kordatou et al., 2015).

2.1.2. Colloids

Colloids are often defined as particles in the size range between 1 nm and 1 µm; they include clays such as layered silicates, metal oxides such as Fe- and -Al- hydroxides, organic material such as humic acids, proteins, and bio-colloids such as bacteria, viruses, and are ubiquitous in the environment (Zhou et al., 2007). Colloids are formed and transported to surface waters by weathering and biological processes. Their large specific surface area and sorption capacity play a crucial role in the speciation, bioavailability and transport of substances in the aquatic environment (Xing et al., 2015; Zhou et al., 2007).

2.1.3. Suspended solids and sediments

Suspended solids are a source of material that, in the case of contaminant loads, becomes a medium for accumulation in the sediment compartment (Lahti and Oikari 2011). Sediments are defined as settled material derived from weathering, erosion of minerals, and decay of organic matter, and transported by the action of the wind, water

or ice, and the force of gravity acting on the suspended solids. Sediments provide an important environmental surface for partitioning processes; and therefore, are of importance in determining the fate of chemicals in the environment.

2.1.4. Importance of sorption in risk assessment

Sorption processes are important in determining predicted environmental concentrations for environmental risk assessments. For example, the OSPAR convention states that compounds with distribution coefficient (log K_{ow}) above 4.5 are screened for persistence, bioaccumulation and toxicity, following European Technical Guidance on Risk Assessment (European Comission Joint Research Centre, 2003). The European Medicines Agency guidelines for the environmental risk assessment of pharmaceutical compounds proposes a Phased approach. Phase 1, a pre-screening worst-case scenario, considers the parent compound only, irrespective of mode of administration, metabolism and excretion (EMA, 2006). If the predicted environmental concentration is above 0.01 $\mu g\ L^{-1}$, Phase 2 entails analysis of the environmental fate and effects, although a tailored environmental risk assessment could be triggered below this threshold if the pharmaceutical is shown to affect reproduction. A PBT assessment is required at Phase 1 for APIs that have a Log K_{ow} >4.5. Phase 2 is a tiered phase, comprising Tiers A and B. Tier A involves the ready biodegradability test (OECD, 1992) followed by a water-sediment distribution study if the analyte is shown to be persistent (OECD, 2000). Toxicity tests are also performed to allow for a Predicted No Effect Concentration to be estimated. If the "Predicted Environmental Concentration" : "Predicted No Effect Concentration" ratio indicates a risk, a Tier B assessment should be conducted where the predicted environmental concentration for surface water is refined to include information

from wastewater treatment plant modelling using the SimpleTreat model (EMA, 2006), where a factor is applied to allow for the partitioning of the chemical.

A terrestrial environmental risk assessment is triggered at Tier B if the K_{oc} of the active pharmaceutical ingredient is >10,000. Fish bioconcentration studies at Tier B are required if the Log K_{ow} >3 and a sediment toxicity assessment is required for pharmaceuticals where 10% of the applied radioactivity adsorbs to sediment in the water-sediment transformation study conducted at Tier A (EMA, 2006).

The distribution of a APIs between the particulate and dissolved phases is therefore critical in determining its environmental fate, including mobility, persistence, bioavailability and subsequent toxicity. It is also critical for defining whether a terrestrial or sediment based environmental risk assessment is required as part of the marketing application in Europe. There is currently no study that compiles the available partitioning data for APIs, nor identifies the significant data gaps and relates it to pharmaceuticals environmental fate. This review evaluates, for the first time, published information regarding the partitioning of APIs to DOM, colloids, suspended solids and sediments in natural surface aquatic environments and relates this to risk assessment processes.

2.2.Meta-analyses

2.2.1. Data collection

Published studies on the distribution of APIs in freshwater environments were screened for the collection of data, documenting the source and concentration of the sorbents used in the experiments, pH, and pharmaceutical partition coefficients along with physicochemical parameters such as pK_a and log K_{ow}. Data sources included the

open chemistry database PubChem (https://pubchem.ncbi.nlm.nih.gov/), available scientific literature and environmental fate estimation program software (EPI Suite™, Table 1, Table 2, Table 3, Table 4).

Figure 6 shows a graphical comparison of the number of articles, pharmaceuticals, metabolites and transformation products, and compounds per functionality investigated for each sorbent. Nine articles about sorption processes of pharmaceuticals to DOM, mostly reference materials but also some wastewater-derived DOM (Table 1), were identified (Bai et al., 2008; Carmosini and Lee, 2009; Ding et al., 2013; Kim et al., 2010; Lützhøft et al., 2000; Maoz and Chefetz, 2010; Martínez-Hernández et al., 2014; Peng et al., 2014; Yamamoto et al., 2003).

The total number of pharmaceuticals studied was 20, comprising 1 neutral species, 3 acidic, 3 basic, 13 zwitterions, and 3 metabolites. The experimental pH ranged from 3 to 8, which is a critical parameter regarding the charge present on the pharmaceutical molecule and its subsequent fate.

Four articles related to the partitioning of pharmaceuticals to colloids; extracted from samples of river water, groundwater, seawater and wastewater (Table 2) (Holbrook et al. 2004; Maskaoui et al. 2007; J. L. Zhou et al. 2007; Maskaoui and Zhou 2010). Here, 10 compounds were studied comprising 2 neutral, 3 acidic, and 5 basic species, with experimental pH ranging from 6.4 to 8.5.

Fourteen articles referring to suspended solids and sediment partitioning were identified, with 70 compounds studied (Al-Khazrajy and Boxall, 2016; Githinji et al., 2011; Lahti and Oikari, 2011; Li et al., 2015; Lin et al., 2010; Löffler et al., 2005; Martínez-Hernández et al., 2014; Maskaoui and Zhou, 2010; Paul et al., 2014; Ra et al.,

2008; Stein et al., 2008; Svahn and Bjorklund, 2015; Yamamoto et al., 2009; Zhou and Broodbank, 2014). Of the compounds studied, 4 were metabolites and 8 were transformation products; 20 were acidic, 38 basic, 7 neutral, and 3 were zwitterions across a pH range of 3.5 to 10 (Table 3, Table 4).

Suspended solids and sediments dominated the studies on pharmaceuticals (70), where a number of metabolites and transformation products were also investigated. Basic compounds were most studied in suspended solids and sediments (38), followed by the acidic (20), neutral compounds (7), and zwitterions (3). The distribution to colloids was investigated for 5 basic, 3 acidic and 2 neutral compounds. The DOM distribution studies included the largest amount of zwitterion compounds (13), followed by acidic (3), basic (3) and neutral (2).

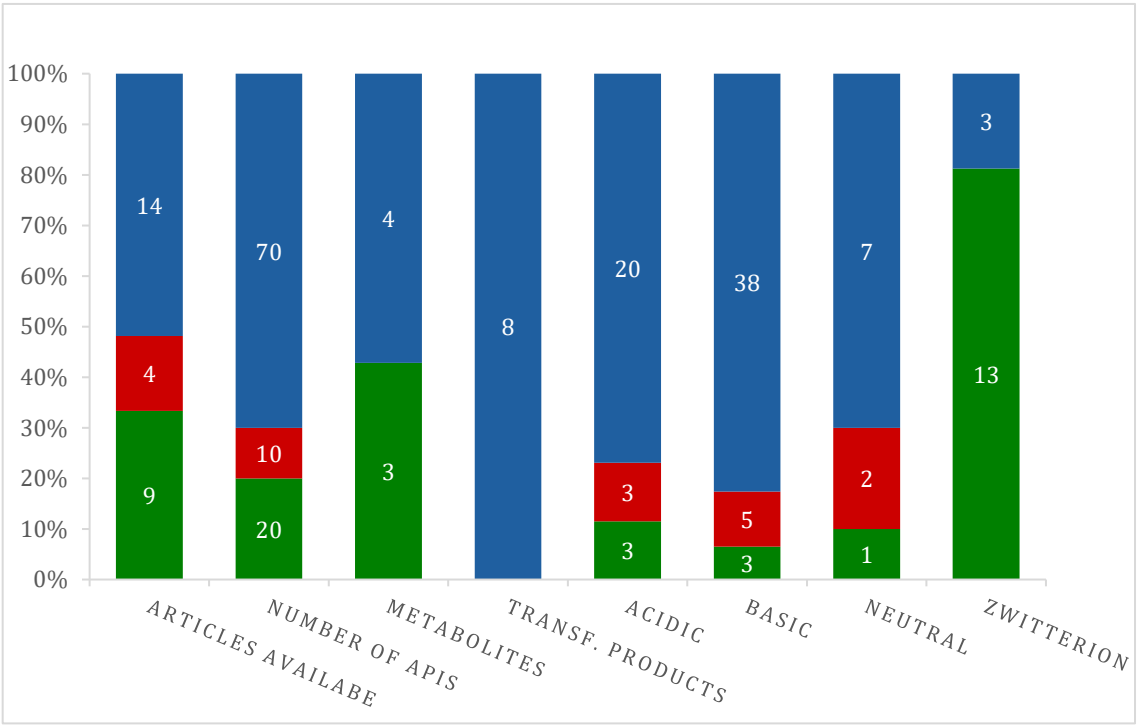


Figure 6 A comparison of published articles on pharmaceuticals partitioning to DOM (green), colloids (red), suspended solids and sediments (blue). For each column is the number of articles, number of studied active

pharmaceutical ingredients (APIs), metabolites, transformation products, and divided per pharmaceuticals functionality: acidic, basic, neutral and zwitterion.

The measured organic carbon-water distribution values (K_{oc}) of each compound were plotted against the corresponding octanol-water distribution coefficient values (K_{ow}) for the three sorbents; i.e. DOM, colloids, and suspended solids and sediments. No relationship was apparent which deviates from correlations for non-polar organics. However, selecting only the neutral pharmaceuticals and plotting organic carbon normalised log K_{oc} against corresponding log K_{ow} for each sorbent did show a linear relationship for DOM (R^2=0.846), albeit for a limited number of data (n=5). No significant relationship was observed for other sorbents (Figure 7).

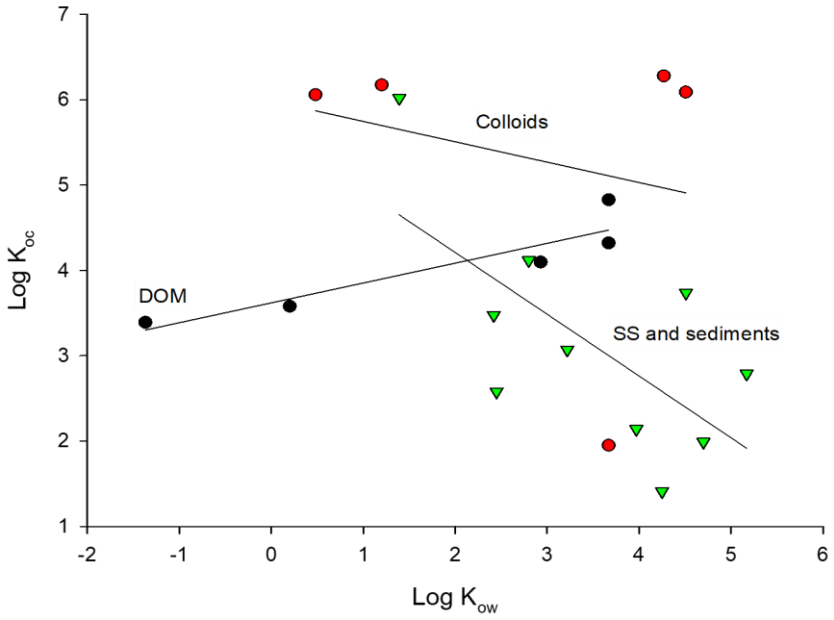


Figure 7 Organic carbon-water (Koc) distribution values of the neutral compounds plotted against the correspondent octanol-water (Kow) distribution coefficient value for each sorbent, namely dissolved organic matter (DOM) (black dots) R2=0.846, colloids (red dots) R2=0.055, suspended solids and sediments (green triangles) R2=0.450; Showing a relationship only for DOM, though for a very limited number of data (5).

2.2.2. Partitioning to dissolved organic matter

Yang et al., (2011) reported a weak positive linear relationship (R^2= 0.509) between the freshwater total concentration of five groups of antibiotics and DOM. Furthermore, Lützhøft et al. (2000) demonstrated an inverse relationship between the concentration of "free" pharmaceuticals in solution and DOM concentration, limiting bioavailability and possibly toxicity (Rowett et al., 2016; Urrestarazu Ramos et al., 1998), but increasing environmental persistence. These two studies highlight both the lack of available data as well as a potentially significant role for DOM in determining environmental exposure to pharmaceuticals.

2.2.2.1. Dissolved organic matter composition

The composition of DOM is source-dependent. For example, anthropogenic wastewater is mainly composed of proteinaceous matter, whilst freshwater DOM is dominated by humic substances (Hudson et al. 2007; Hernandez-Ruiz et al. 2012). The partitioning of the β-blocker, propranolol, on DOM derived from river and lake surface water generated log K_{dom} values ranging from 3.9 - 5.2 L kg^{-1} (Table 1), with protein-like DOM associated with lower values and humic-like substances exhibiting highest log K_{dom} (Peng et al., 2014). Similar results were obtained for the anti-epileptic drug, carbamazepine, which showed most extensive partitioning to humic substances, including fulvic acids from landfill leachate, river water and Amherst humic acid, with log K_{dom} between 3.41 and 5.04 L kg^{-1} (Table 1) (Hernandez-Ruiz et al., 2012a). However, complexation of carbamazepine with tryptophan-like DOM was also significant, suggesting an important role for anthropogenic organic matter in the transport and transformation of such pharmaceuticals (Wang et al., 2016). Since carbamazepine is

a neutral compound, hydrophobic sorption mechanisms to humic acid were proposed (Bai et al. 2008).

2.2.2.2. Organic matter reference standards as a proxy for partitioning of pharmaceuticals

The use of reference standards of humic DOM as a proxy for measuring partitioning of pharmaceuticals to DOM may result in an overestimation of the binding capacity. Carmosini and Lee, (2009) and Peng et al., (2014) investigated the partitioning of the zwitterion, ciprofloxacin, to contrasting DOM types at different pH and ionic strengths. The DOM included Leonardite standard humic acid, Pahokee Peat humic acid, Pahokee Peat II standard fulvic acid and Elliott Soil II standard fulvic acid. Other DOM types were obtained from digested and undigested municipal biosolids, municipal wastewater effluent, and beef industry lagoon wastewater. The wastewater-derived DOM showed much less affinity for ciprofloxacin than the reference humic standards. Furthermore, the former did not show a pH-sorption correlation as in the latter DOM type, which suggests that a mechanism other than ion exchange was controlling the sorption process. These interactions are likely driven by the more aliphatic protein composition of the wastewater-derived DOM than the aromatic humic DOM of the reference standards, exhibiting a different sorption characteristic and cation exchange capacity. Such variation in partitioning has also been observed for pharmaceuticals, carbadox, tetracycline, and lincomycin, tested using reference standards such as Leonardite humic acid and Aldrich humic acid, where the former showed greatest sorption capacity, probably as a result of more interaction sites being available (Table 1) (Ding et al., 2013).

Table 1 Dissolved organic matter (DOM) partition coefficient (Log KDOM) for pharmaceuticals, sources and type of organic matter, experimental concentration and pH. Legend: * metabolites; N.A. not available.

Compound	Type	pKa	Log K_{ow}	Source of DOM	DOM type	Concentration [mg L^{-1}]	pH	Log K_{DOM} [L kg^{-1}]	Reference	Year
17α – ethynylestradiol	acidic	10.21	3.67	Reference standards; river;	Aldrich humic acid	2, 10	7	4.78	Yamamoto et al.	2003
					Suwannee river humic acid	2, 10	7	4.80	Yamamoto et al.	2003
					Suwannee river fulvic acid	2, 10	7	4.55	Yamamoto et al.	2003
					Nordic fulvic acid	2, 10	7	4.63	Yamamoto et al.	2003
					Alginic acid	2, 10	7	3.23	Yamamoto et al.	2003
					Dextran	2, 10	7	3.04	Yamamoto et al.	2003
					Tannic acid	2, 10	7	5.22	Yamamoto et al.	2003
Albendazole	zwitterion	3.37, 9.93	3.07	Reference standards; synthesis;	Aldrich humic acid	5000-15000	7	3.52	Kim et al.	2010
					hydroxypropyl-b-cyclodextrin	20000	7	2.96	Kim et al.	2010
					POPC liposome	480-1700	7	3.77	Kim et al.	2010
					sodium dodecyl sulfate	10000-25000	7	3.02	Kim et al.	2010
Amino-fenbendazole	Zwitterion	6.94, 10.65	3.17	Reference standards; synthesis	Aldrich humic acid; hydroxypropyl-	5000-15000	7	n. a.	Kim et al.	2010
					b-cyclodextrin; sodium dodecyl					
					sulfate; 1-palmitoyl-2-oleoyl-sn-glycero-					
					3-phosphocholine; lyposome;					
Carbadox	Basic	1.98	n. a.	Reference standards;	Leonardite	46.4	8	3.52	Ding et al.	2013
					Humic acids	79.4	8	3.26	Ding et al.	2013
Carbamazepine	Neutral	13.9	2.93	Landfill leachate	FA	25	7	3.41 to 5.04	Bai et al.	2008
				Amherst humic acid	HA	25	7	4.58 - 4.82	Bai et al.	2008

				Wastewater and Suwannee river.	n. a.	8	n. a.	n. a.	Hernandez -Ruiz et al.	2012
				Sewage sludge	Bulk DOM	1000	8	2.64	Maoz and Chefetz	2010
Ciprofloxacin	Zwitterion	5.9; 8.9	0.4	Digested and undigested biosolids; treated wastewater; beef lagoon wastewater; humic acids reference standards;	≤1000 Da; ≥1000 Da fraction;	25	n. a.	n. a.	Carmosini and Lee	2009
Fenbendazole	Zwitterion	5.12, 12.72	3.85	Reference standards; synthesis;	Aldrich humic acid	5000-15000	7	3.06	Kim et al.	2010
					hydroxypropyl-b-cyclodextrin	20000	7	3.25	Kim et al.	2010
					POPC liposome	480-1701	7	3.23	Kim et al.	2010
					sodium dodecyl sulfate	10000-25000	7	2.92	Kim et al.	2010
Fenbendazole sulfone*	Zwitterion	3.41, 11.12	2.17	Reference standards; synthesis;	Aldrich humic acid; hydroxypropyl-	5000-15000	7	n. a.	Kim et al.	2010
					b-cyclodextrin; sodium dodecyl					
					sulfate; 1-palmitoyl-2-oleoyl-sn-glycero-					
					3-phosphocholine ; lyposome;					
Flubendazole	Zwitterion	3.6, 9.6	2.91	Reference standards; synthesis;	Aldrich humic acid	5000-15000	7	2.60	Kim et al.	2010
					hydroxypropyl-b-cyclodextrin	20000	7	2.08	Kim et al.	2010
					POPC liposome	480-1702	7	n.a.	Kim et al.	2010
					sodium dodecyl sulfate	10000-25000	7	2.04	Kim et al.	2010
Flumequine	Zwitterion	6.4	1.72	Reference standard;	Aldrich Humic acid	1, 5, 12.5, 25, 50	3	3.44	Lützhøft et al.	2000
						1, 5, 12.5, 25, 50	4	3.65	Lützhøft et al.	2000
						1, 5, 12.5, 25, 50	5	4.23	Lützhøft et al.	2000
						1, 5, 12.5, 25, 50	6	4.39	Lützhøft et al.	2000
						1, 5, 12.5, 25, 60	7	4.23	Lützhøft et al.	2000
						1, 5, 12.5, 25, 50	8	4.36	Lützhøft et al.	2000
Ibuprofen	Acidic	4.41	3.5	Wastewater and Suwannee river.	n. a.	8	n. a.	n. a.	Hernandez -Ruiz et al.	2012
Lincomycin	Basic	7.6	0.2	Reference standard	Leonardite humic acid	46.4	8	3.96	Ding et al.	2013

					Aldrich humic acid	79.4	8	3.20	Ding et al.	2013
Naproxen	Acidic	4.2	3.18	Sewage sludge	Hydrophobic acid, basic, and neutral fractions; hydrophilic acid, basic, and neutral fractions.	1000	8	negligible	Maoz and Chefetz	2010
Oxfendazole*	Zwitterion	4.13, 11.79	1.63	Reference standards; synthesis;	n. a.	5000-15000	7	n. a.	Kim et al.	2010
Oxolinic acid	Zwitterion	6.9	0.68	Aldrich humic acids;	Humic acids;	1, 5, 12.5, 25, 50	3	3.87	Lützhøft et al.	2000
						1, 5, 12.5, 25, 50	4	4.00	Lützhøft et al.	2000
						1, 5, 12.5, 25, 50	5	4.37	Lützhøft et al.	2000
						1, 5, 12.5, 25, 50	6	4.50	Lützhøft et al.	2000
						1, 5, 12.5, 25, 50	7	4.48	Lützhøft et al.	2000
						1, 5, 12.5, 25, 50	8	4.37	Lützhøft et al.	2000
p-hydroxyfenbendazole*	Zwitterion	5.49, 9.48, 11.38	3.37	Reference standards; synthesis;	n. a.	5000-15000	7	n. a.	Kim et al.,	2010
Propranolol	Basic	9.7	n. a.	River and lake surface–water filtered DOM; sediment-extracted DOM;	Humic acids; Fulvic acids; pony lake fulvic acid;	10	7	3.90/5.20	Peng et al.	2014
					Suwannee river fulvic acid;	10	7	4.70	Peng et al.	2014
Sarafloxacin	Zwitterion	n. a.	n. a.	Aldrich humic acids;	Humic acids;	1, 5, 12.5, 25, 50	3	4.77	Lützhøft et al..	2000
					Humic acids;	1, 5, 12.5, 25, 50	4	4.86	Lützhøft et al.	2000
					Humic acids;	1, 5, 12.5, 25, 50	5	5.19	Lützhøft et al.	2000
					Humic acids;	1, 5, 12.5, 25, 50	6	4.98	Lützhøft et al.	2000
					Humic acids;	1, 5, 12.5, 25, 50	7	4,74	Lützhøft et al.	2000
					Humic acids;	1, 5, 12.5, 25, 50	8	4.52	Lützhøft et al.	2000
Tetracycline	Zwitterion	3.3; 7.7; 7.9	n. a.	Reference standards	Leonardite humic acid	46.4	8	4.60	Ding et al.	2013
					Aldrich humic acid	79.4	8	3.27	Ding et al.	2013
Thiabendazole	Zwitterion	4.7, 12	2.47	Reference standards; synthesis;	Aldrich humic acid	5000-15000	7	2.58	Kim et al.	2010
					hydroxypropyl-b-cyclodextrin	20000	7	2.39	Kim et al.	2010

POPC liposome	480-1703	7	n. a.	Kim et al.	2010
sodium dodecyl sulfate	10000-25003	7	2.01	Kim et al.	2010

2.2.2.3. Hydrophobicity of dissolved organic matter and its impact on partitioning

The role of the different DOM fractions, namely hydrophobic or hydrophilic, and the sub-classes acidic, basic or neutral, on partitioning of pharmaceuticals depends on their chemical functionalities and degree of ionisation (Lützhøft et al., 2000; Michael-Kordatou et al., 2015). For example, hydrophobic DOM fractions dominate the distribution of the acidic naproxen and the neutral carbamazepine at low pH (4), whilst at more environmentally relevant pH (6.5 - 8), the hydrophilic DOM fractions were more influential (Maoz and Chefetz 2010). In fact, despite the predominance of the acidic fraction of the hydrophilic DOM, which is expected to show little affinity for the negatively-charged naproxen, there is evidence that the neutral and basic moieties play an important role in the binding of the neutral carbamazepine and the acidic ibuprofen (Hernandez-Ruiz et al., 2013). Notwithstanding, the contribution to binding of each fraction is determined by its relative abundance in DOM mixtures (Maoz and Chefetz 2010). The calculated values regarding sorption of pharmaceuticals to bulk DOM are based on experimentally discrete fractional sorption values, and differ from bulk experimental measurements (Maoz and Chefetz 2010). On this basis, it is hypothesised that DOM fractions interact with each other at the molecular level and do not behave as independent entities in the binding processes. Thus, studying DOM fractions as discrete

entities may lead to incorrect estimates of pharmaceuticals partitioning to the bulk DOM (Maoz and Chefetz 2010).

2.2.3. Partitioning to colloids

The partitioning of pharmaceuticals to colloids is poorly understood, with little supporting research available. The available data show that pharmaceuticals partition to colloids to a greater degree than other sorbents (Figure 8) (Table 2). The sorption capacity of colloids is determined by the type, influenced by its source and environmental compartment, such as freshwater, wastewater, or seawater. Such a variation can lead to large differences in partition coefficient values for the same pharmaceutical (Zhou et al., 2007). Examples include data for colloids from river water, treated effluent, and seawater showing a distribution coefficient variation of a factor of 6 - 12 for the synthetic oestrogen 17-α ethynylestradiol. In addition, despite a higher amount of colloidal fraction in the wastewater effluent, no major partitioning variation was observed between wastewater effluents and freshwater colloids, whilst higher K_{coc} values were observed for the lower concentrations of seawater colloids. This data confirms that the physical-chemical properties of the colloids have much more influence than their actual concentration on the distribution of pharmaceuticals (Zhou et al., 2007).

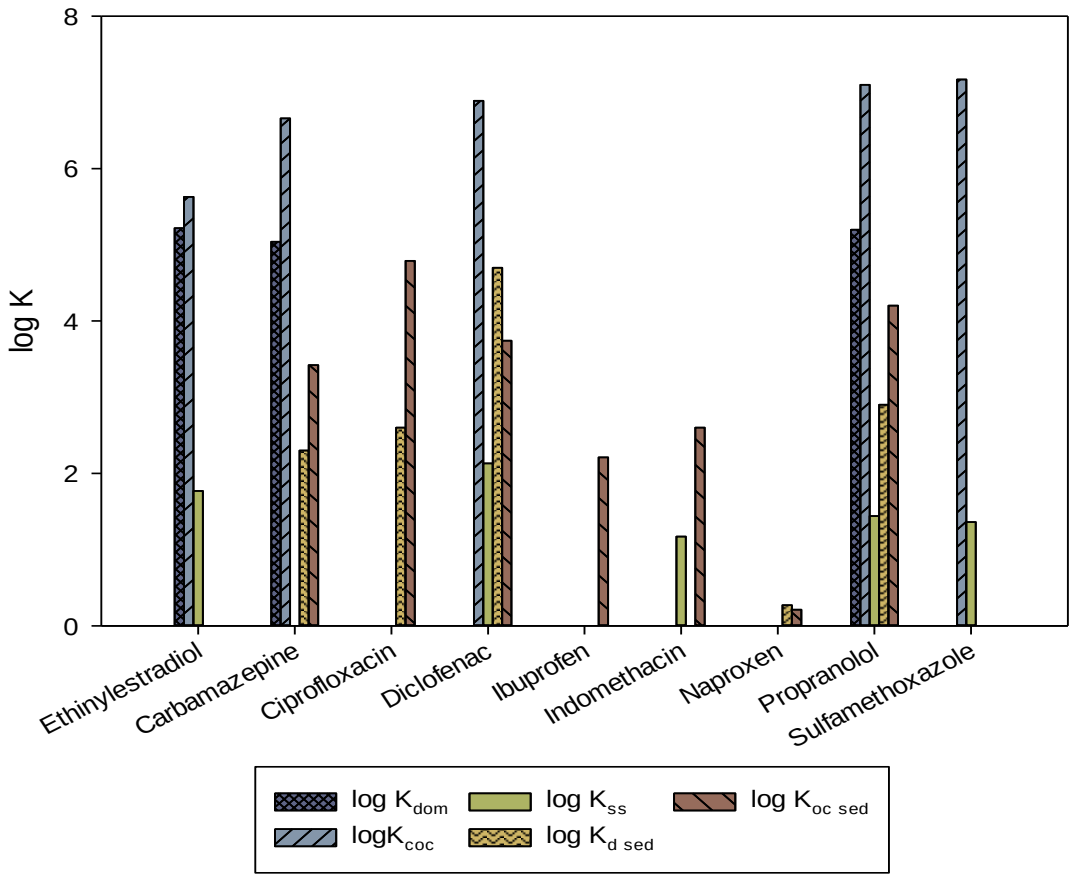


Figure 8 Comparison of pharmaceuticals partition coefficients for different sorbents, namely dissolved organic matter (log K_{dom}), colloids (log K_{coc}), suspended solids (log K_{ss}), and sediments -bulk (log K_d sed)) and -normalized per organic matter fraction (log K_{oc} sed)).

Table 2 Colloids partition coefficients (Log Kcoc) for pharmaceuticals, source and size fraction, experimental concentration and pH. Legend: * metabolites; N.A. not available.

Compound	Type	pKa	Log K_{ow}	Source	Size fraction	Concentration (mg L^{-1})	pH	Log K_{coc} (L kg^{-1})	Reference	Year
17α-Ethynilestradiol	neutral	10.2	3.67	Biological wastewater treatment system	<30kD	6.0	7.1	5.2	Holbrook et al.	2004
				Biological wastewater treatment system	<30kD	5.3	7.1	3.0	Holbrook et al.	2004
				Biological wastewater treatment system	<30kD	4.9	7.1	5.2	Holbrook et al.	2004
				Biological wastewater treatment system	<30kD	5.1	7.1	4.5	Holbrook et al.	2004
				Biological wastewater treatment system	<100kD	5.9	7.1	4.7	Holbrook et al.	2004
				Biological wastewater treatment system	<100kD	5.4	7.1	4.5	Holbrook et al.	2004
				Biological wastewater treatment system	<0.22 µm	7.7	7.1	5.4	Holbrook et al.	2004
				Biological wastewater treatment system	<0.22 µm	7.5	7.1	4.9	Holbrook et al.	2004
				Biological wastewater treatment system	<0.22 µm	5.5	7.1	5.0	Holbrook et al.	2004
				Biological wastewater treatment system	<0.22 µm	7.1	7.1	5.3	Holbrook et al.	2004
				Biological wastewater treatment system	<1.5µm	8.9	7.1	4.8	Holbrook et al.	2004
				Biological wastewater treatment system	<1.5µm	7.7	7.1	5.0	Holbrook et al.	2004
				Biological wastewater treatment system	<1.5µm	6.2	7.1	4.9	Holbrook et al.	2004
				Biological wastewater treatment system	<1.5µm	7.8	7.1	5.2	Holbrook et al.	2004
				River water	between >1 kDa and <0.7 µm;	2.3	6.4	4.57	Zhou et al.	2007
				River water	between >1 kDa and <0.7 µm;	3	7.3	3.8	Zhou et al.	2007
				STWs effluents	between >1 kDa and <0.7 µm;	9.2	7.1	3.73	Zhou et al.	2007
				River water	between >1 kDa and <0.7 µm;	2.9	8.5	3.84	Zhou et al.	2007
				Sea water	between >1 kDa and <0.7 µm;	0.4	8	4.47	Zhou et al.	2007
				STWs effluents	between >1 kDa and <0.7 µm;	7.5	6.8	4.63	Zhou et al.	2007
Carbamazepine	neutral	13.94	2.93	River water; STWs effluents; groundwate;			n. a.	6.66	Maskaoui and Zhou	2010
				River water	between >1 kDa and <0.7 µm;	0-30	n. a.	4.74	Maskaoui, Hibberd, & Zhou	2007
Diclofenac	acidic	4	4.51	River water; STWs effluents; groundwater;			n. a.	6.89	Maskaoui and Zhou	2010

				River water	between >1 kDa and <0.7 µm;	0-30	n. a.	5.29	Maskaoui, Hibberd, & Zhou	2007
Indomethacin	acidic	3.96	4.27	River water; STWs effluents; groundwater;			n. a.	7.06	Maskaoui and Zhou	2010
				River water	between >1 kDa and <0.7 µm;	0-30	n. a.	5.5	Maskaoui, Hibberd, & Zhou	2007
Mebeverine	basic	8.2	5.12	River water; STWs effluents; groundwater;			n. a.	n. a.	Maskaoui and Zhou	2010
				River water	between >1 kDa and <0.7 µm;	0-30	n. a.	5.88	Maskaoui, Hibberd, & Zhou	2007
Meclofenamic acid	acidic	3.73	5	River water; STWs effluents; groundwater;			n. a.	n. a.	Maskaoui and Zhou	2010
				River water	between >1 kDa and <0.7 µm;	0-30	n. a.	5.29	Maskaoui, Hibberd, & Zhou	2007
Propranolol	basic	9.7	1.2	River water; STWs effluents; groundwater;			n. a.	7.1	Maskaoui and Zhou	2010
				River water	between >1 kDa and <0.7 µm;	0-30	n. a.	5.25	Maskaoui, Hibberd, & Zhou	2007
Sulfamethoxazole	basic	6.2	0.48	River water; STWs effluents; groundwater;			n. a.	7.17	Maskaoui and Zhou	2010
				River water	between >1 kDa and <0.7 µm;	0-30	n. a.	4.95	Maskaoui, Hibberd, & Zhou	2007
Tamoxifen	basic	8.87	6.3	River water; STWs effluents; groundwater;	n. a.	n. a.	n. a.	n. a.	Maskaoui and Zhou	2010
Thioridazine	basic	9.5	5.9	River water; STWs effluents; groundwater;			n. a.	n. a.	Maskaoui and Zhou	2010

Published data show no relationship between K_{oc} and K_{ow} for a series of APIs (Holbrook et al., 2004; Liu et al., 2005; Maskaoui et al., 2007; Yamamoto et al., 2003), which has been related to mechanisms of sorption dictated chiefly by the charge on the pharmaceuticals rather than hydrophobicity (Holbrook et al., 2004; Yamamoto et al., 2003). Nevertheless, it should be noted that Maskaoui et al. (2007) showed a positive relationship between K_{coc} and K_{ow}, and postulated a role for hydrophobicity in controlling the partitioning processes of APIs on colloids. However, the study did not present the experimental pH and the variability of the charge of the compounds studied. The pK_as suggest that most of the acidic and basic compounds would have been dissociated at environmental pH, which would strongly influence the relationship between K_{ow} and K_{coc}.

2.2.4. Partitioning to suspended solids & sediments

Preferential partitioning of some pharmaceuticals to suspended solids, and their absence in surface water solution in river catchments, highlights the significance of sorbents (Ferreira Da Silva et al., 2011). Both the characteristics of the pharmaceutical and the suspended solids are important in determining observed behaviour; in this case, sorption was driven by the positively-charged functionality of the pharmaceutical (Ferreira Da Silva et al., 2011) (Table 3).

Table 3 Suspended solids partition coefficients (Log K_d) and normalized to organic matter content (log K_{oc}) for pharmaceuticals, source, sorbent, experimental concentration and pH. Legend: * metabolites; N.A. not available.

Compound	Type	pKa	Log K_{ow}	Source	Sorbent	Concentration ($mg\ L^{-1}$)	pH	Log K_d ($L\ Kg^{-1}$)	Log K_{oc} [$L\ Kg^{-1}$]	Reference	Year
17α-Ethynilestradiol	neutral	10.2	3.67	Synthetic	Artificial alumina suspended particles coated with humic acid;	1	7.8	1.77	3.93	Ra et al.	2008
Amoxicillin	Zwitter ion	2.8; 7.2	0.87	Synthetic wastewater	Suspended solids	160	3.5	0.41	n. a.	Githinji et al.	2011
							5.5	0.55	n. a.	Githinji et al.	2011
							6.6	0.64	n. a.	Githinji et al.	2011
							7.5	0.80	n. a.	Githinji et al.	2011
							8.5	1.08	n. a.	Githinji et al.	2011
Ciprofloxacin	Zwitter ion	5.9; 8.9	0.28	Synthetic wastewater	Suspended solids	160	3.5	-0.17	n. a.	Githinji et al.	2011
							5.5	-0.05	n. a.	Githinji et al.	2011
							6.6	-0.07	n. a.	Githinji et al.	2011
							7.5	-0.21	n. a.	Githinji et al.	2011
							8.5	-0.36	n. a.	Githinji et al.	2011
Diclofenac	acidic	4	4.51	River water; STWs effluents;	Suspended solids	n. a.	n. a.	0.95	n. a.	Maskaoui and Zhou	2010
				Synthetic	Artificial alumina suspended particles coated with humic acid;	1	7.8	2.13	4.29	Ra et al.	2008
Gemfibrozil	acidic	4.5	4.7	Synthetic	Artificial alumina suspended particles coated with humic acid;	1	7.8	2.39	4.55	Ra et al.	2008
Ibuprofen	acidic	4.41	3.5	Synthetic wastewater	Suspended solids	n. a.	3.5	-2.77	n. a.	Paul et al.	2013
				Synthetic wastewater	Suspended solids	n. a.	6.5	-2.96	n. a.	Paul et al.	2013
				Synthetic	Alumina suspended particles coated with humic acid	1	7.8	1.59	3.76	Ra et al.	2008
Indomethacine	acidic	3.96	15	River water; STWs effluents;	Suspended solids	n. a.	n. a.	1.17	n. a.	Maskaoui and Zhou	2010
Mebeverine	neutral	8.2	5.12	River water; STWs effluents;	Suspended solids	n. a.	n. a.	n. a.	n. a.	Maskaoui and Zhou	2010
Meclofenamic acid	acidic	3.73	5	River water; STWs effluents;	Suspended solids	n. a.	n. a.	n. a.	n. a.	Maskaoui and Zhou	2010
Naproxen	acidic	4.2	3.18	Synthetic wastewater	Suspended solids	n. a.	3.5	-4.52	n. a.	Paul et al.	2013

							n. a.	6.5	-3.14	n. a.	Paul et al.	2013
Propranolol	basic	9.7	1.2	River water; effluents;	STWs	Suspended solids	n. a.	n. a.	1.44	n. a.	Maskaoui and Zhou	2010
Sulfamethoxazole	basic	9.14; 13.84	0.48	River water; effluents;	STWs	Suspended solids	n. a.	n. a.	1.36	n. a.	Maskaoui and Zhou	2010
Tamoxifen	basic	8.87	6.3	River water; effluents;	STWs	Suspended solids	n. a.	n. a.	n. a.	n. a.	Maskaoui and Zhou	2010
Thioridazine	basic	9.5	5.9	River water; effluents;	STWs	Suspended solids	n. a.	n. a.	n. a.	n. a.	Maskaoui and Zhou	2010
Tolfenamic acid	acidic	4.3	5.17	Synthetic		Artificial alumina suspended particles coated with humic acid;	1	7.8	2.79	4.95	Ra et al.	2008

There are limited data on pharmaceutical sediment sorption, accumulation, and formation of transformation products (Bu et al., 2013; Savci, 2013) (Table 4). One study reported concentrations 10 – 32 times higher in sediments than in water (Agunbiade and Moodley, 2016) with other studies confirming the importance of sediments as sorbents (Stein et al. 2008; Yamamoto et al. 2009; Varga et al. 2010; Martínez-Hernández et al. 2014; Svahn and Bjorklund 2015; Li et al. 2015). For acidic pharmaceuticals, however, the charge repulsion limits pharmaceutical - sediment interactions (Koumaki et al., 2016).

Table 4 Sediments partition coefficients (Log Kd) and normalized to organic matter content (log K_{oc}) for pharmaceuticals, source, and pH. Legend: * metabolites; N.A. not available.

Compound	Type	pKa	Log K_{ow}	Source	pH	Log K_d (L Kg^{-1})	Log K_{oc} (L Kg^{-1})	Reference	Year
10,11-dihydro-10,11-dihydroxycarbamazepine*	n. a.	n. a.	n. a.	River	7.7	-0.52	1.46	Loffler et al.	2005
				River	n. a.	-0.8-0.25	1.3-1.6	Stein et al.	2008
10,11-dihydrocarbamazepine*	n. a.	n. a.	n. a.	Settleable particulate matter from wastewater treatment works effluents	n. a.	n. a.	n. a.	Lahti and Oikari	2011
				River	n. a.	0.08-0.94	2.2-2.3	Stein et al.	2008
1-Naphthol+	neutral	9.6	2.7	Lake	6.8-8	n. a.	n. a.	Li et al.	2015

2-Hydroxyibuprofen+	acidic	4.6	2.4	Lake	6.8-9	n. a.	n. a.	Li et al.	2015
				River	7.7	n. a.	n. a.	Loffler et al.	2005
4-Amino-6-chloro-1,3-benzenedisulfonamide+	basic	9.2	-1	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
4-chlorobenzoic acid+	acidic	4.1	2.2	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
4'-Hydroxydiclofenac+	acidic	3.8	4	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Acebutolol	basic	9.2	1.71	Settleable particulate matter from wastewater treatment works effluents	n. a.	0.5-1.0	2.35-2.47	Lahti and Oikari	2011
				River		3.28	5.05	Lin et al.	2010
Acetaminophen	neutral	9.5	0.91	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
				River	n. a.	-0.3	0.75	Martínez-Hernández et al.	2014
				River	n. a.	0.41-1-0.44	4.11-2.43-5.4	Yamamoto et al.	2009
				River		0.7	2.47	Lin et al.	2010
Alprenolol	basic	n. a.	n. a.	Settleable particulate matter from wastewater treatment works effluents	n. a.	n. a.	n. a.	Lahti and Oikari	2011
Amitryptyline	basic	9.4	4.92	River	7	0.94-2.39	2.95-4.10	Al-Khazrajy and Boxall	2016
Atenolol	basic	9.6	0.16	Settleable particulate matter from wastewater treatment works effluents	n. a.	0.05-0.5	1.85-2.05	Lahti and Oikari	2011
				River	n. a.	0.9	-0.25	Martínez-Hernández et al.	2014
				River, lake	n. a.	n. a.	n. a.	Svahn and Bjorklund	2015
				River		0.11-0.9-0.72	3.23-2.92-2.5	Yamamoto et al.	2009
				River	7	0.34-1.31	1.93-2.7	Al-Khazrajy and Boxall	2016
Bendroflumethiazide	basic	n. a.	n. a.	River, lake		n. a.	n. a.	Svahn and Bjorklund	2015
Bezafibrate	acidic	3.6	4.25	Settleable particulate matter from wastewater treatment works effluents	n. a.	-0.5	1.41	Lahti and Oikari	2011
				Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Bicalutamide	basic	12	2.7	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Bisoprolol hemifumarate	basic	9.5	1.87	Settleable particulate matter from wastewater treatment works effluents	n. a.	0.3-0.8	2.17-2.3	Lahti and Oikari	2011
Caffeine	basic	6.1	<0	River		1.25	-0.8	Martínez-Hernández et al.	2014
				River		2.4	4.16	Lin et al.	2010
Carbamazepine	neutral	13.9	2.45	Settleable particulate matter from wastewater treatment works effluents	n. a.	0.2-2.3	2.00-3.42	Lahti and Oikari	2011
				Lake	6.8-10	n. a.	n. a.	Li et al.	2015
				River	7.7	0.11	1.92	Loffler et al.	2005

				River		-0.39	1.8	Martínez-Hernández et al.	2014
				River		0.23-1.09	2.4-2.5	Stein et al.	2008
				River, lake		n. a.	n. a.	Svahn and Bjorklund	2015
				River		-1.07-0.14-0.25	1.04-2.14-2	Yamamoto et al.	2009
				River		n. a.	2.6-3.7	Zhou and Broodbank	2014
Carbamazepine-10,11-epoxide+	neutral	16	2	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Carboxyibuprofen	acidic	4	2.8	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Chlorothiazide	basic	9.1	-0.44	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Chlortalidone	basic	8.6	1.6	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Cimetidine	basic	6.8	0.40	River	7	0.35-1.20	2.00-2.63	Al-Khazrajy and Boxall	2016
Ciprofloxacin	zwitterion	5.9-8.9	0.4	Settleable particulate matter from wastewater treatment works effluents	n. a.	2.6	4.79	Lahti and Oikari	2011
Citalopram	basic	9.6	1.39	Settleable particulate matter from wastewater treatment works effluents	n. a.	3.9-4.6	5.32-6.02	Lahti and Oikari	2011
Clofibric acid	acidic	3.4	2.9	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
				River	7.7	-0.52	1.41	Loffler et al.	2005
Codeine	basic	8.2	1.2	River		0.32-1.15	2.4-2.5	Stein et al.	2008
D3-ibuprofen	acidic	n. a.	n. a.	Settleable particulate matter from wastewater treatment works effluents	n. a.	n. a.	n. a.	Lahti and Oikari	2011
D5-fluoxetine	basic	n. a.	n. a.	Settleable particulate matter from wastewater treatment works effluents	n. a.	n. a.	n. a.	Lahti and Oikari	2011
Demeclocycline	basic	n. a.	n. a.	Setteable particulate matter from wastewater treatment works effluents	n. a.	n. a.	n. a.	Lahti and Oikari	2011
Diazepam	basic	3.4	2.85	River	7.7	0.48	2.28	Loffler et al.	2005
				River	n. a.	0.28-1.4	2.4-2.8	Stein et al.	2008
Diclofenac	acidic	4.2	4.51	Settleable particulate matter from wastewater treatment works effluents	n. a.	4.7	2.45-3.74	Lahti and Oikari	2011
				Lake	6.8-10	n. a.	n. a.	Li et al.	2015
				River, lake		n. a.	n. a.	Svahn and Bjorklund	2015
				River	n. a.	1.58 to 2.71	n. a.	Zhou and Broodbank	2014
Dihydrocodeine*	basic	8.8	-1.5	River		0.15-0.81	2.3-2.2	Stein et al.	2008
Diltiazem	basic	8.06	2.8	River	7	1.34-3.00	2.90-4.12	Al-Khazrajy and Boxall	2016
Enrofloxacin	acidic	n. a.	n. a.	Settleable particulate matter from wastewater treatment works effluents	n. a.	n. a.	n. a.	Lahti and Oikari	2011
Fluconazole	basic	13	0.56	Lake	6.8-10	n. a.	n. a.	Li et al.	2015

Fluoxetine	basic	10.1	1.22	Settleable particulate matter from wastewater treatment works effluents	n. a.	2.9-4.1	4.09-5.49	Lahti and Oikari	2011
				River		1.25-2.7-3.63	4.38-4.7-5.4	Yamamoto et al.	2009
Furosemide	acidic	4.2	1.8	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
				River, lake		n. a.	n. a.	Svahn and Bjorklund	2015
Glimepiride	basic	4.3	3.1	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Hydrochlorothiazide	basic	9.1	-0.58	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Ibuprofen	acidic	4.9	3.97	Settleable particulate matter from wastewater treatment works effluents	n. a.	1.7	2.14-2.21	Lahti and Oikari	2011
				Lake	6.8-10	n. a.	n. a.	Li et al.	2015
				River		7.7	n. a.	Loffler et al.	2005
				River		-1.03- -0.04- -0.52	2.07- 1.97 - 1.25	Yamamoto et al.	2009
Ifenprodil	basic	9.34, 9.99	n. a.	River		1.5 - 2.66 - 3.14	4.61 - 4.67 - 4.91	Yamamoto et al.	2009
Indomethacin	acidic	3.96	n. a.	River		-0.92 - 0.17 - 0.83	2.20 - 2.20 - 2.60	Yamamoto et al.	2009
Iopromide	basic	n. a.	-2.33	River		7.7	n. a.	Loffler et al.	2005
Ivermectin	neutral	n. a.	3.22	River		7.7	3.07	Loffler et al.	2005
Ketoprofen	acidic	4.5	3.12	Settleable particulate matter from wastewater treatment works effluents	n. a.	n. a.	n. a.	Lahti and Oikari	2011
				Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Mefenamic acid	acidic	3.73	2.42	River		1.30-0.74-1.08	4.43- - 0.38-2.85	Yamamoto et al.	2009
				River	7	0.26-1.27	1.88-2.52	Al-Khazrajy and Boxall	2016
Metoprolol	basic	9.7	1.69	Settleable particulate matter from wastewater treatment works effluents	n. a.	0.2-0.9	2.22-2.24	Lahti and Oikari	2011
				Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Metoprolol acid+	acidic	3.5	-1.2	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Morphine	basic	8	-0.1	River		0.5-1.33	12.6-2.7	Stein et al.	2008
N4-acetyl-sulfamethoxazole*	basic	n. a.	n. a.	River		-1.88- - 0.15	0.24-1.2	Stein et al.	2008
Naproxen	acidic	4.2	3.18	Settleable particulate matter from wastewater treatment works effluents	n. a.	n. a.	n. a.	Lahti and Oikari	2011
				Lake	6.8-10	n. a.	n. a.	Li et al.	2015
				River		0.27	0.21	Martínez-Hernández et al.	2014
Ofloxacin	zwitterion	6.0, 8.3	0.35	Suspended particulate matter from wastewater treatment works effluents	n. a.	2.5	4.64	Lahti and Oikari	2011
Oxazepam	basic	1.7	2.4	Suspended particulate matter from wastewater treatment works effluents	7.7	0.34	2.18	Loffler et al.	2005

				River		0.30-1.37	2.4-2.7	Stein et al.	2008
				River, lake		n. a.	n. a.	Svahn and Bjorklund	2015
Oxytetracycline	basic	3.3, 7.3, 9.1	-1.22	Suspended particulate matter from wastewater treatment works effluents	n. a.	2.5	4.64	Lahti and Oikari	2011
Paracetamol	basic	9.4	0.46	Suspended particulate matter from wastewater treatment works effluents	n. a.	n. a.	n. a.	Lahti and Oikari	2011
				Suspended particulate matter from wastewater treatment works effluents	7.7	n. a.	n. a.	Loffler et al.	2005
Propranolol	basic	9.7	2.6	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
				River sediment		0.34-2-2.20	3.46-4-3.97	Yamamoto et al.	2009
				River		2.43	4.2	Yu-Chen Lin et al.	2010
				River		1.89 to 2.9	3.11-3.95	Zhou and Broodbank	2014
Saluamine	acidic	4.4	0.66	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Sotalol	basic	9.6	0.24	Suspended particulate matter from wastewater treatment works effluents	n. a.	0.1-0.6	1.94-2.15	Lahti and Oikari	2011
				Lake	6.8-10	n. a.	n. a.	Li et al.	2015
Sulfamethoxazole	acidic	6.2	0.9	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
				Aquifer		0.63	-0.17	Martínez-Hernández et al.	2014
				River		-0.7- -0.05	1.4-1.3	Stein et al.	2008
				River		1.41 to 2.67	n. a.	Zhou and Broodbank	2014
				River		0.75-1.44	2.19-2.8	Stein et al.	2008
Tramadol	basic	2.4	9.2	Lake	6.8-10	n. a.	n. a.	Li et al.	2015
				River		0.38-0.89	3.01-2.5	Stein et al.	2008
α-Hydroxymetoprolol+	basic	0.84	9.7	Lake		n. a.	n. a.	Li et al.	2015

2.2.4.1. Influence of compound speciation

The degree of ionisation influences partitioning on suspended solids (Table 3), with strong pH dependence for zwitterions, such as ciprofloxacin and amoxicillin, controlling partitioning to suspended solids in wastewater (Githinji et al., 2011). Acidic ibuprofen and naproxen were adsorbed in larger amounts at pH lower than their pK_a (4.4 and 4.2, respectively), where they were mainly in unionized form (Paul et al., 2014).

In contrast, for zwitterions, an increase in K_d was observed at lower pH due to protonation of the amine group. As might be expected, higher K_d values were observed for amine-containing pharmaceuticals than for neutral or carboxylic compounds (Martínez-Hernández et al., 2014; Stein et al., 2008; Svahn and Bjorklund, 2015; Yamamoto et al., 2009) (Table 4). For example, ibuprofen showed a significant increase in K_d as pH decreased from 7 to 4, the latter pH value being below the pK_a of the acidic compound (4.5), meaning that the neutral species dominated (Oh et al., 2016). Stein et al. (2008) showed that compounds with structures similar to carbamazepine and its metabolites, and some opiates and tranquilizers, had a similar distribution trend. Furthermore, the dominance of ionization over hydrophobicity as a sorption mechanism to suspended solids and sediments was confirmed by poor correlation of K_d and K_{ow} (Stein et al., 2008; Yamamoto et al., 2009).

2.2.4.2. Sediment composition

The importance of the sediment composition to sorption was demonstrated by a large variation in K_d using field data from varying sampling locations (Zhou and Broodbank, 2014). The organic carbon content of the sediment correlated with the degree of pharmaceutical sorption (Martínez-Hernández et al., 2014; Stein et al., 2008; Svahn and Bjorklund, 2015; Varga et al., 2010), which is consistent with the large K_{oc} values reported (Table 4). Despite the important role played by the organic carbon fraction, variability in cation exchange capacity and sediment texture can also influence sorption of APIs (Al-Khazrajy and Boxall, 2016; Le Guet et al., 2018). Furthermore, the molecular weight of pharmaceuticals was found to be positively correlated with K_{oc}, showing that the partitioning processes tended to favour relatively large molecules, similar to hydrophobic contaminants (Zhou and Broodbank, 2014). Resuspension of sediment leads

to a decrease in K_d (Aminot et al., 2015; Zhou and Broodbank, 2014). This is likely due the larger specific surface area of suspended solids compared with sediments, leading to a shift in equilibrium towards the more readily available exchange sites of the suspended solids.

2.2.4.3. Accumulation in sediments

The sorption of APIs onto sediments and resuspendable suspended solids may lead to accumulation. APIs contamination of the benthic environment in areas close to urban wastewater treatment works, with concentrations up to 200 ng g^{-1} dry weight, has been reported (Lahti and Oikari 2011). Once in the sediment, APIs may undergo transformation processes and release lower molecular weight, more soluble transformation products into the dissolved phase which, in turn, partition according to the physico-chemical properties of the newly-formed moieties. Thus, sediments have been considered a potential secondary source of pharmaceutical contamination, likely to occur over a wide area downstream of discharges and therefore should be recognized as part of the environmental risk caused by pharmaceuticals in the environment (Li et al. 2015).

2.2.4.4. Metabolite sorption

In addition to the parent compounds, sorption of metabolites is little understood, with only three studies having included metabolic products (Lahti and Oikari, 2011; Löffler et al., 2005; Stein et al., 2008). Löffler et al. (2005) compared the sorption to sediments of 10 pharmaceuticals and metabolites, including diazepam and its metabolite oxazepam; ibuprofen and its metabolite 2-hydroxyibuprofen; carbamazepine and its metabolite 10,11-dihydro-10,11-dihydroxycarbamazepine, metabolite and the active

form of clofibrate of clofibric acid, iopromide, paracetamol, and ivermectin. Binding to sediments was ascribed by the authors as the main reason for rapid elimination of paracetamol from solution. Carbamazepine showed a moderate affinity for sediments, whilst the accumulation of 10,11-dihydro-10,11-dihydroxycarbamazepine was insignificant, most likely because of its high solubility and hydrophilicity (log K_{ow} = 0.13) (Miao et al., 2005). The lipid regulator, clofibric acid, showed a very low affinity for sediments at pH close to its pK_a of 7.7, suggesting low sorption under most environmental conditions. Due to its moderate hydrophobicity, oxazepam was rapidly and extensively partitioned to sediments (Löffler et al., 2005). The high hydrophobicity of ivermectin was reported as the main cause of rapid and extensive sorption to sediments. Ibuprofen partitioned to sediments only moderately, as did its metabolite 2-hydroxyibuprofen. In summary, sorption to sediments was elevated for ivermectin, diazepam, oxazepam, and carbamazepine with K_{oc} ranging from 1172 L kg^{-1} for ivermectin to 83 L kg^{-1} for carbamazepine (Löffler et al., 2005), consistent with their respective physico-chemical properties (Table 4).

2.2.5. Implication for environmental risk assessments

In the environmental risk assessment for `down-the-drain chemicals', such as pharmaceuticals, water is the main environmental compartment of concern (EMA, 2006). As such, the distribution to DOM, colloids, suspended solids and sediments in surface fresh water are of importance in determining the environmental exposure of these contaminants. The sorption to freshwater sorbents is accounted for in the predicted environmental concentration refinement of Tier B of the environmental risk assessment for pharmaceuticals by the calculation of a factor, as shown in Equation 2 (EMA, 2006; European Comission Joint Research Centre, 2003):

Equation 2

$$FACTOR = \left(1 + Kp_{susp} * SUSP_{water} * 10^{-6}\right) \quad 2$$

Where Kp_{susp} is the distribution coefficient to suspended solids; and $SUSP_{water}$ the concentration of suspended solids.

However, this calculation considers sorption on suspended solids but not specifically on DOM and/or colloidal fractions. As DOM and colloidal matter are responsible for most sorption (Figure 8), their omission could lead to overestimation of exposure. Additionally, as evidenced from this study, the source and quality of the sorbent is a key factor in determining the partitioning extent of pharmaceuticals. This is particularly apparent when reference standards are used to assess the degree of sorption to DOM, and results in a high degree of uncertainty of the distribution capacity. These aspects should be addressed in site-specific risk assessment as they could profoundly affect the exposure estimate. The importance of the pH dissociation of the pharmaceuticals is also clearly demonstrable, which impacts significantly on the extent of sorption to freshwater sorbents.

2.3. Conclusions

As a general conclusion, there is a paucity of information on processes by which APIs sorb to surfaces in aquatic environments. Nevertheless, reports show that the sorption of pharmaceuticals in the aquatic environment strongly depends on their chemical functionality and the sorbent properties, with basic compounds more readily adsorbed than neutral or acidic ones. Therefore, sorption increases at pH below pK_a for all functionalities. The comparison of the partition coefficients of the different sorbents

(Figure 8) shows a net predominance of sorption onto colloids, and the following general trend of sorption: colloids > DOM > sediments > suspended solids. The K_{ow} does not demonstrate a relationship with the distribution coefficients (K) of the polar and ionizable pharmaceuticals and the analyzed sorbents, which is most likely due to additional polar interactions between ionized pharmaceutical functional groups and the sorbent. Also, the adjustment of K to pH has not established a relationship between the adjusted partition coefficient (D) and K_{ow}.

With regard to DOM composition, humic substances generally show more affinity for APIs than protein-like DOM, while a poor correlation was found between sorption of pharmaceuticals reference and to environmental DOM. Furthermore, it is important to note that the investigation of the role of different fractions of DOM in isolation does not represent an environmentally-realistic estimate of pharmaceutical sorption. Partitioning to colloids is significantly under-researched, with the limited available information suggesting they are a major physico-chemical variable controlling pharmaceutical partitioning in water. Concerning other identified sorbents, suspended solids interactions with pharmaceuticals show dependence on the degree of pharmaceutical ionization. Again, no relationship between K_{ow} and K_{dss} was demonstrated. Sediments can be considered both as a sink for pharmaceuticals and as a secondary source of pharmaceutical contamination in the form of transformation products. Differences in sediment K_d from separate studies are most likely influenced by differences in sediment composition, e.g. organic carbon content, grain size, clay fraction, cation exchange capacity, experimental conditions, and the concentration of pharmaceutical used, which can vary from mg L^{-1} in experimental conditions to ng L^{-1}.

3. MATERIALS AND METHODS

3.1. Introduction

The aim and objectives of this research were addressed through a series of laboratory experiments and a final field study. In this chapter are described the materials, such as the active pharmaceuticals ingredients (APIs) and the synthetic wastewater (SW). A description of the sample preparation (i.e. filtering and solid phase extraction (SPE)) and the development of the High Pressure Liquid Chromatography (HPLC) coupled with Mass Spectrometry (MS) methodology is also described, as well as the fluorescence spectrophotometry and total organic carbon (TOC) analysis techniques.

All the laboratory scale experiments were conducted at the University of Plymouth. The fieldwork consisted of a sampling campaign carried out in the Nairobi/Athi River Catchment, Kenya, and in such case the samples preparation was conducted in the laboratory of Jomo Kenyatta University, Nairobi, whilst the analyses were partly performed at the University of Plymouth and partly at the University of York. All the study specific materials and methods are outlined in the respective relevant chapters.

3.2. APIs selection

The APIs for the research needed to be representative for LLMICs where DDUW is occurring with greatest impact. Also the API functionality was considered as a selection criterion owing to its importance in distribution processes. Accounting for these two criteria substances were selected on the basis of the abundance of information in the literature, and the evidence of occurrence in LLMICs. The first parameter was considered necessary because of the novelty of the research. The latter parameter was deemed

necessary for extending the research to compounds typically found in impact zones of LLMICs.

Table 5 provides a list of the selected APIs, namely the neutral carbamazepine (CBZ), acetaminophen (ACE), and nevirapine (NVR), the acidic diclofenac (DCF) and valsartan (VLS), the basic acetobutol (ACE) and amitriptyline (AMI); and their structure, functionality, pKa, log K_{ow}, molecular formula, monoisotopic mass, and charged mass - positive and negative.

All the compounds were purchased at the highest purity available either from Sigma-Aldrich (Acetobutol hydrochloride, amitriptyline hydrochloride, nevirapine, valsartan, acetaminophen) or Fisher Scientific (carbamazepine, diclofenac sodium).

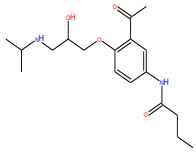
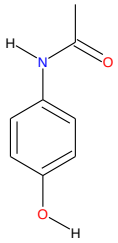
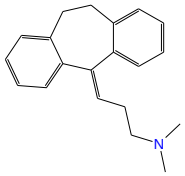
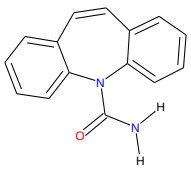
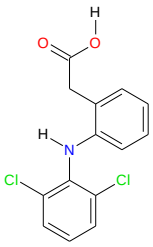
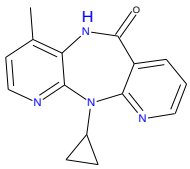
3.1.1. Acebutolol

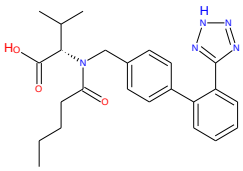
ACE is a cardio-selective beta blocker for the treatment of hypertension and arrhythmias. It reduces the work of the heart and allows it to beat more regularly, which is valid for other beta blockers as well (Drugbank, 2018a).

The DDD[1] for ACE is of 0.4 g via oral or parental administration e.g. by injection or intravenously, according to the World Health Organization (WHO, 2018a). The elimination of ACE is approximately of the 30-40% via renal excretion and the 50-60% via non-renal mechanisms, including excretion through the bile and direct passage through the intestinal wall (Drugbank, 2018a).

[1] "The basic definition of the defined daily dose (DDD) is the assumed average maintenance dose per day for a drug used for its main indication in adult. Drug utilization data presented in DDDs only give a rough estimate of consumption and not an exact picture of actual use." (WHO, 2018g).

Table 5, list of the selected active pharmaceutical ingredients (APIs), namely acebutolol (ACE), acetaminophen (ACT), amitriptyline (AMI), carbamazepine (CBZ), diclofenac (DCF), nevirapine (NVR) and valsartan (VLS), and their structure, functionality, pKa, log K_{ow}, molecular formula, monoisotopic mass and charged mass: positive and negative.

APIS	STRUCTURE	FUNCTIONALITY	PKA	LOG K_{OW}	MOLECULAR FORMULA	MONOISOTOPIC MASS	[M+H]+	[M-H]-
ACE		Basic	9.2	1.71	$C_{18}H_{28}N_2O_4$	336.2049	337.2121	335.1976
ACT		Neutral	9.38	0.34	$C_8H_9NO_2$	151.0633	152.0706	150.0560
AMI		Basic	9.4	4.92	$C_{20}H_{23}N$	277.1830	278.1903	276.1757
CBZ		Neutral	13.9	2.67	$C_{15}H_{12}N_2O$	236.0949	237.1022	235.0876
DCF		Acidic	4	4.06	$C_{14}H_{11}Cl_2NO_2$	295.0166	296.0239	294.0094
NVR		Neutral	2.8	2.5	$C_{15}H_{14}N_4O$	266.1167	267.1240	265.1094

VLS		Acidic	4.7; 3.9	3.65	$C_{24}H_{29}N_5O_3$	435.2270	436.2343	434.2197

3.1.2. Acetaminophen

ACT, also known as paracetamol, is a pharmaceutical widely used as analgesic and antipyretic. The therapeutic effects of ACT are close to the salicylates but lacking of anti—inflammatory, antiplatelet and gastric ulcerative effects (Drugbank, 2018b).

The DDD for ACT is of 3 g via the oral, parental administration e.g. by injection or intravenously, or rectal according to the World Health Organization (WHO, 2018b). ACT undergoes mainly glucoronidation (45-55% of the dose) and sulfation (30-35% of the dose). About 80% of ACT is excreted in urine after transformation and the 3% only unchanged (Drugbank, 2018b).

This API was chosen for his widespread use and as a relatively well studied compound, which therefore allows its use as a reference compound. The physico-chemical properties of ACT are listed in Table 5.

3.1.3. Amitriptyline

AMI is used in the treatment of depression and as an analgesic. This compound has a strong effect on the mood of depressed individuals through the inhibition of serotonin and norepinephrine reuptake (Drugbank, 2018c).

The DDD of AMI is of 75 mg via the oral, parental administration e.g. by injection or intravenously (WHO, 2018c). AMI is mainly metabolized as the active nortriptyline in the liver, which can undergo further hydroxylation followed by conjugation with glucoronic acid. Other metabolites are formed but with much weaker activity (Drugbank, 2018c). AMI is mostly excreted through urine as glucuronide or sulphate conjugate of

metabolites, and only 2% the total amount in urine is its parental form; a small amount is also detectable in faeces (Drugbank, 2018c).

AMI was chosen because of its high prescription rate and its detection in the environment (Li et al., 2013), and it was considered of interest in a mechanistic study of sorption processes for its relatively high distribution coefficient (log K_{ow} 4.92) and its basic functionality (Table 5).

3.1.4. Carbamazepine

CBZ is an anticonvulsant used to control epileptic seizure. The mechanisms of action are not fully understood yet (Drugbank, 2018d).

The DDD of CBZ is of 1 g via the oral, parental administration e.g. by injection or intravenously (Drugbank, 2018d). CBZ is excreted mainly in urine (72%) and the rest is found in faeces (28%). The parental compound constitutes only the 3% of the total (Drugbank, 2018d).

This API was chosen because of it has been relatively well studied and therefore well-known environmental behaviour, which made it the ideal candidate as control compound. The physico-chemical properties of CBZ are furnished in Table 5.

3.1.5. Diclofenac

DCF is a non-steroidal anti-inflammatory drug used for its antipyretic and analgesic action in the treatment of acute and chronic signs and symptoms of osteoarthritis and rheumatoid arthritis and pain more in general (Drugbank, 2018e).

The DDD for DCF is of 0.3 g via the oral, parental administration e.g. by injection or intravenously, or rectal according to the World Health Organization (WHO, 2018d). DCF is excreted totally in its glucuronide and sulphate conjugates of the metabolites in urine (65%) and only a little fraction of the total as conjugate of the parent compound and metabolites through biliary excretion (35%) (Drugbank, 2018e).

DCF was chosen as relatively well studied compound and global wide use, in addition to its proved ecotoxicological effects (Swan et al., 2006). Also, the negatively charged functional group at environmental relevant pH was considered of interest for mechanistic studies of distribution conducted in this research.

3.1.6. Nevirapine

NVR is a widely used antiretroviral with activity against human immunodeficiency virus type 1 (HIV-1). NVR is prescribed when the symptoms of the decline of the immune system have become evident and infections are spreading. The virus can become resistant to NVR if used without other HIV medications, and it is effective only for a limited time (Drugbank, 2018f).

The DDD for NVR is of 0.4 g via the oral administration only according to the World Health Organization (WHO, 2018e). This API is extensively metabolised via the cytochrome P450 metabolism in the liver. Only a small fraction of the parent compound is excreted through urine (<3%), indicating a minor role of such excretion route (Drugbank, 2018f).

This compound was chosen as widely prescribed and detected in LLMICs in Africa (K’oreje et al., 2012; Madikizela et al., 2017; Ngumba et al., 2016) and for its

resemblance of physico-chemical properties to the highly persistent CBZ, which suggested a similar behaviour in the environment (Table 5).

3.1.7. Valsartan

VLS is an angiotensin-receptor blocker used to treat a variety of cardiac conditions such as hypertension, diabetic nephropathy and heart failure. VLS belongs to a class of antihypertensive agents called angiotensin II receptor blockers (ARBs) (Drugbank, 2018g).

The DDD for VLR is of 80 mg via the oral administration only according to the World Health Organization (WHO, 2018f). This compound is excreted 80% as unchanged drug and is not metabolised significantly in humans. The main excretion route is through faeces (83%) and the 13% is through urine, mainly as the parent compound (Drugbank, 2018g).

VLS was chosen as a widely used API in high and LLMICs (Siddiqui et al., 2011). Additionally, it was chosen as representative for negatively charged compounds (Table 5).

3.1.8. Others

In addition to these compounds a list of 55 APIs was screened for in the samples from a field work conducted in the Nairobi/Athi River catchment in Kenya. These compounds are listed in the relevant chapter as it is out of the scope of this section to give a detailed description of every single API screened in such work.

3.3. Synthetic wastewater and characterization

This section is based on the supporting information of the paper published as: Bagnis, S., Fitzsimons, M., Snape, J., Tappin, A., & Comber, S. (2018). Sorption of active pharmaceutical ingredients in untreated wastewater effluent and effect of dilution in freshwater: Implications for an "impact zone" environmental risk assessment approach. Science of the Total Environment, 624, 333–341. https://doi.org/10.1016/j.scitotenv.2017.12.092.

The use of synthetic wastewaters (SW) is widely adopted for research purposes and in operational management. For instance in laboratory or pilot-scale WWTW where the use of an artificial wastewater is aimed at the control of loading conditions (O'Flaherty and Gray, 2013). SW are typically used for testing the fate of chemical, for studies of compounds toxicity, for the design, evaluation and optimisation of treatment technologies, and for the investigation of biological treatment processes (Githinji et al., 2011; Paul et al., 2014). There are several reasons for adopting a SW instead than real wastewater. The first reason is that SW can provide reproducibility because of the constant composition in terms of carbon and nutrients, in contrast to real wastewater; secondly, the use of 'real' wastewater may not be feasible or practical (i.e. there may be no WWTW near the laboratory); thirdly, the use of real wastewater can lead to an occupational risk caused by the pathogenic bacterial population likely to be present in the wastewater; In addition, the synthetic formulation is not as malodorous as real wastewater (O'Flaherty and Gray, 2013).

There are many SW formulations described in the literature. The Organization for Economic Co-operation and Development (OECD) series of guidelines for testing

of chemicals proposes a SW that has been widely used for regulatory purposes. Due to the statutory nature of the OECD, the relative guidelines for biodegradation tests are often considered as the "confirmatory test" when the use of different guidelines can lead to variation in the results (O'Flaherty and Gray, 2013). In these guidelines the use of a SW has been commonly referred as the 'standard SW' since it is routinely used in such tests (O'Flaherty and Gray, 2013; OECD, 2001). Because of the importance given to this formulation, and the wide acceptance as reference standard SW, there has been a reluctance to use any other. However, this formulation has not been exempt from criticisms. Boeije et al. (1999) criticise that the use of peptone and meat extract in the OECD method can lead to operational problems such as sludge bulking, and blame the unbalanced composition as a possible cause of decline of microbial activity, in comparison with real domestic sewage. Another criticism regards the biological nutrient removal and the unsuitability of the medium owing to an unbalanced ratio between C/N/P, respectively 100:18:2 (Dubber and Gray, 2011). Regardless of the OECD formulation, more general criticisms consider SWs as not representative of real wastewaters because of incomparability of COD and C/N/P ratios to real wastewaters, which can lead to bacterial growth selectivity and therefore different microbial population composition than the one present in WWTWs (Boeije et al., 1999). Despite all the criticisms, it must be emphasized that there is not a standard real wastewater; Metcalf and Eddy (2003) provide a table with a range of data for a typical untreated domestic wastewater composition, which may be adopted as a guideline (Table 6) (Tchobanoglous et al., 2003).

A review of SW recipes available in literature was conducted by O'Flaherty and Gray (2013). The review aims at the identification of the most realistic

composition by means of comparison with real wastewaters samples. As already mentioned, the synthetic SW formulation mostly used refers to the OECD guidelines (OECD 303A). The OECD recipe is a suggestion, and can be modified depending on the experimental conditions the user is seeking to simulate. The original recipe was augmented with 200 mg/L of glucose by Prochaska and Zouboulis (2009); and it was used in a TOC concentration of 10 to 50 mg L^{-1} by Zwiener et al., (2000). The other SW analysed were Bracklow et al., (2007), Chuang et al., (1998), Kiso et al., (2000), La Para et al., (2006), Nopens et al., (2001), Stasinakis et al., (2003), Synthetic sewage 2 (BSI, 2007), Tsang et al., (2008), Syntho (Boeije et al., 1999), and Synthes (Aiyuk and Verstraete, 2004).

No one recipe is completely representative for wastewater, but each could be suitable for a different experimental application. In particular, *Syntho* and *Sinthes* have formulations that achieve the closest resemblance to real wastewater (O'Flaherty and Grey, 2013). A SW formulation involving the use of pet rabbit feed as insoluble organic suspended solids source was developed (Esperanza et al., 2004) and used as wastewater feed in a laboratory scale wastewater treatment plant. The same formulation was adopted afterwards by other authors for sorption studies (Githinji et al., 2011; Paul et al., 2014). This case is mentioned to report the variability in SW formulations.

According to the authors, there is no such a thing as an ideal synthetic wastewater which is appropriate all situations and the criteria for the selection is dependent on the intended application. Regardless, the use of synthetic wastewater provides distinct advantages over the use of real wastewater, especially its reproducibility (O'Flaherty and Gray, 2013).

Table 6 Typical compositions of untreated domestic wastewater (Tchobanoglous et al., 2003).

Constituent	Unit	Concentration		
		Low strength	Medium strength	High strength
Solids, total (TS)	mg L^{-1}	537	806	1612
Dissolved, total (TDS)	mg L^{-1}	374	560	1121
Fixed	mg L^{-1}	224	336	672
Volatile	mg L^{-1}	150	225	449
Suspended solids, total (TSS)	mg L^{-1}	130	195	389
Fixed	mg L^{-1}	29	43	86
Volatile	mg L^{-1}	101	152	304
Settleable solids	mL L^{-1}	8	12	23
Biochemical oxygen demand 5d, 20 ˚C (BOD)	mg L^{-1}	133	200	400
Total organic carbon (TOC)	mg L^{-1}	109	164	328
Chemical oxygen demand (COD)	mg L^{-1}	339	508	1016
Nitrogen (total as N)	mg L^{-1}	23	35	69
Organic	mg L^{-1}	10	14	29
Free ammonia	mg L^{-1}	14	20	41
Nitrites	mg L^{-1}	0	0	0
Nitrates	mg L^{-1}	0	0	0
Phosphorus (total as P)	mg L^{-1}	3.7	5.6	11
Organic	mg L^{-1}	2.1	3.2	6.3
Inorganic	mg L^{-1}	1.6	2.4	4.7
Potassium	mg L^{-1}	11	16	32
Chlorides	mg L^{-1}	39	59	118
Sulfate	mg L^{-1}	24	36	72
Oil and Grease	mg L^{-1}	51	76	153
Volatile organic compounds (VOCs)	µg L^{-1}	<100	100-400	>400
Total coliform	No./100ml	10^{6}-10^{8}	10^{6}-10^{9}	10^{7}-10^{10}
Fecal coliform	No./100ml	10^{3}-10^{5}	10^{4}-10^{6}	10^{5}-10^{8}
***Cryptosporidium* oocysts**	No./100ml	10^{-1}-10^{1}	10^{-1}-10^{2}	10^{-1}-10^{3}

***Giardia lamblia* cysts**	No./100ml	10^{-1}-10^{2}	10^{-1}-10^{3}	10^{-1}-10^{4}

Syntho, one of the synthetic wastewater (SW) recipe available in literature was selected to be used in this study (Boeije et al., 1999). The original recipe was augmented three times in order to obtain a concentration typical for high strength wastewater (Tchobanoglous et al., 2003).

The chemicals used for the synthesis of the SW (Table 7) were: Genapol C100, Potassium molybdate, Genapol X-080 (Sigma Aldrich); Starch, for biochemistry, potato, hydrolysed for electrophoresis, Acros Organics; Glycerol, 99+%, extra pure, Acros Organics; Ammonium chloride; Uric acid, 99+%, Acros Organics; Kieselguhr, pure, white; Sodium bicarbonate, 99.7+%, ACS reagent, Acros Organics; Iron(III) sulphate, technical; Cobalt(II) chloride hexahydrate, for analysis, Acros Organics; Chromium(III) nitrate monohydrate, 99%, Acros Organics; Copper(II) chloride dihydrate, extra pure, SLR; Ethylenediaminetetraacetic acid, extra pure, SLR; manganese(II) sulfate monohydrate, 99+%, extra pure, Acros Organics; nickel(II) sulphate hexahydrate, 99%, for analysis, Acros Organics; zinc chloride, 99.99%, (trace metal basis), anhydrous, Acros Organics; sodium acetate anhydrous, extra pure, SLR; Dextrin, Acros Organics; Lactose monohydrate, Certified AR for analysis, fine powder; Meat Extract (Sigma-Aldrich); Peptone special, for microbiology (Sigma-Aldrich); Urea, analytical regent grade (Fisher); potassium phosphate monobasic (Fisher); magnesium phosphate monobasic (Fisher); calcium chloride dehydrate (Fisher).

Table 7 List of ingredients of Syntho and augmented concentration values (Boeije et al. 1999).

Compound	*Unit*	*Amount*	*Compound*	*Unit*	*Amount*
Na acetate*	mg L^{-1}	100	**Genapol C100*****	mg L^{-1}	10
Dried meat extract***	mg L^{-1}	45	**Lyophilized sludge+**	mg L^{-1}	600
Low-fat milk powder***	mg L^{-1}	100	**$CaCl_2$****	mg L^{-1}	15
Potato starch*	mg L^{-1}	100	**$NaHCO_3$***	mg L^{-1}	75
Glycerol*	mg L^{-1}	60	**$FeSO_4$ $3H_2O$***	mg L^{-1}	30
Peptone***	mg L^{-1}	85	**$CoCl_2$ $6H_2O$***	µg L^{-1}	150
NH_4CL*	mg L^{-1}	33	**$Cr(NO_3)9H_2O$***	µg L^{-1}	2040
Urea**	mg L^{-1}	225	**$CuCl_2$ $2H_2O$***	µg L^{-1}	1440
Uric acid*	mg L^{-1}	27	**EDTA***	µg L^{-1}	0.66
$K_3PO_4H_2O$**	mg L^{-1}	60	**K_2MoO_4*****	µg L^{-1}	60
$MgHPO_4$ $3H_2O$**	mg L^{-1}	75	**$MnSO_4$ H_2O***	µg L^{-1}	300
Genapol X-080***	mg L^{-1}	10	**$NiSO_4$ $6H_2O$***	µg L^{-1}	900
Diatomaceous earth*	mg L^{-1}	30	**$ZnCl_2$***	µg L^{-1}	540
Diet fibres*	mg L^{-1}	100			

*, obtained from Acros organic, UK; ***, obtained from Sigma-Aldrich, UK; **, obtained from Fisher, UK; +, Central Wastewater Treatment Works at Plymouth, Devon, UK.

The excitation-emission fluorescence matrix (EEM) of the SW showed the typical protein-like composition of crude wastewater, namely tryptophan-like (T1) and tyrosine-like (T2) peaks, and a minor peak of humic acids (HA) (Figure 9) (Hernandez-Ruiz et

al., 2012b). Figure 10 shows the evolution of the peaks of the three components of Syntho, namely tyrosine-like, tryptophan-like and humic acids, during the 24 hours. The stability of all the three peaks is achieved after 12 hours. The results from FTIR analysis (Figure 11) showed a chemical "fingerprint" which remained constant, meaning that the medium was stable over the duration of the experiment. The respective curves in the graph were all consistent over time and the precision demonstrates the reproducibility of the SW.

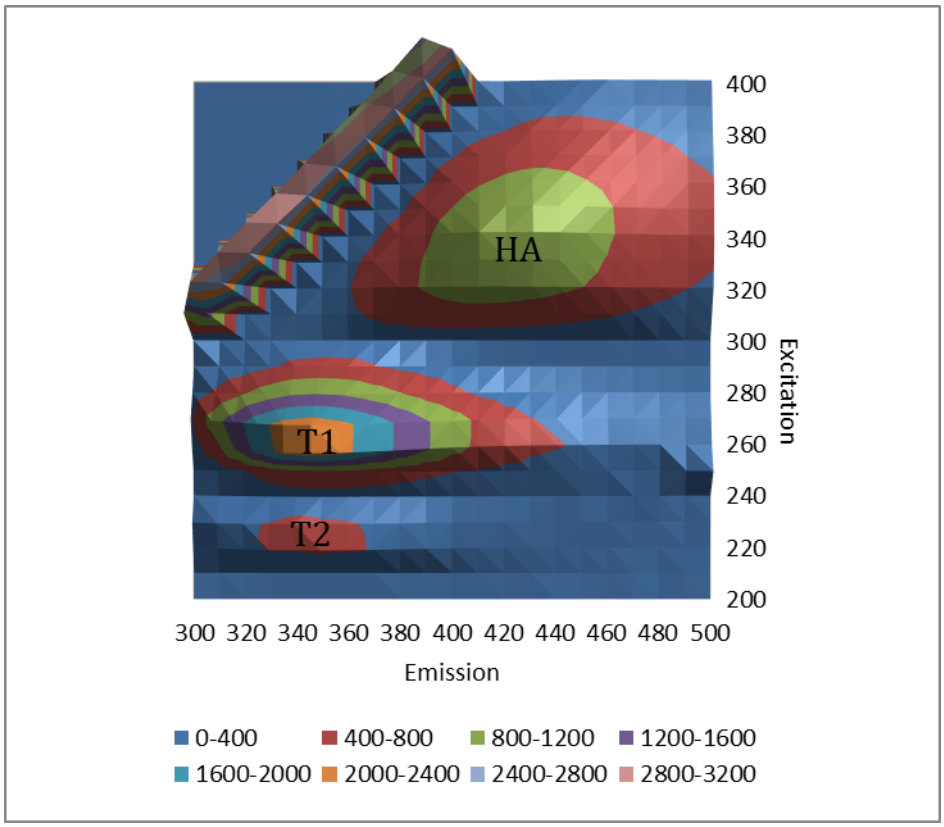


Figure 9 the excitation-emission fluorescence matrix of the synthetic wastewater (Syntho). T1 is the tryptophan-like peak; T2 is the tyrosine-like peak; HA is the humic acids peak. The legend shows the intensity of the peak (dimensionless).

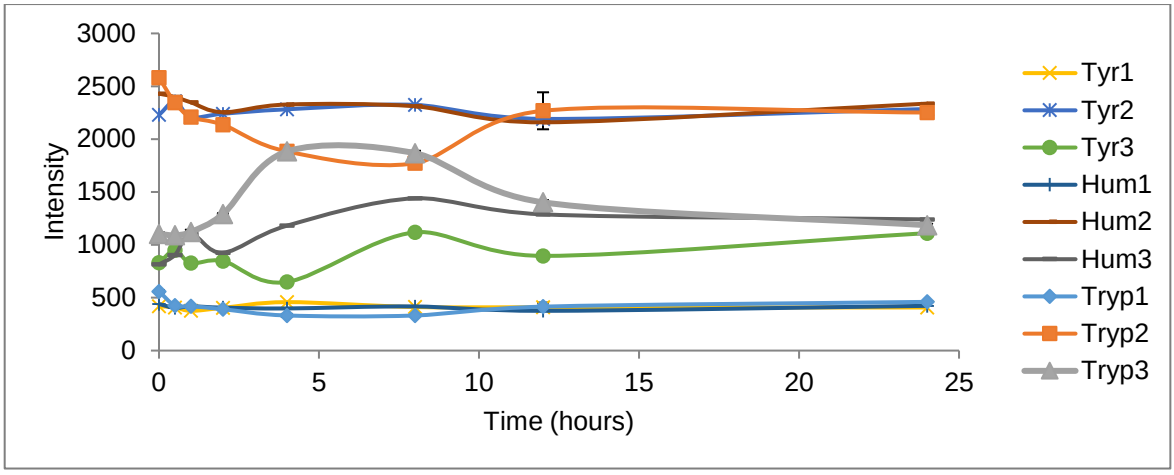


Figure 10 the evolution of the peaks of the three components of Syntho, namely Tyrosine-like, Tryptophan-like and humic acids, during the duration of 24 hours. The stability of all the three peaks is achieved after 12 hours.

In addition the peaks observed for the SW using FTIR showed strong similarities to real wastewater (Chefetz et al., 2006).

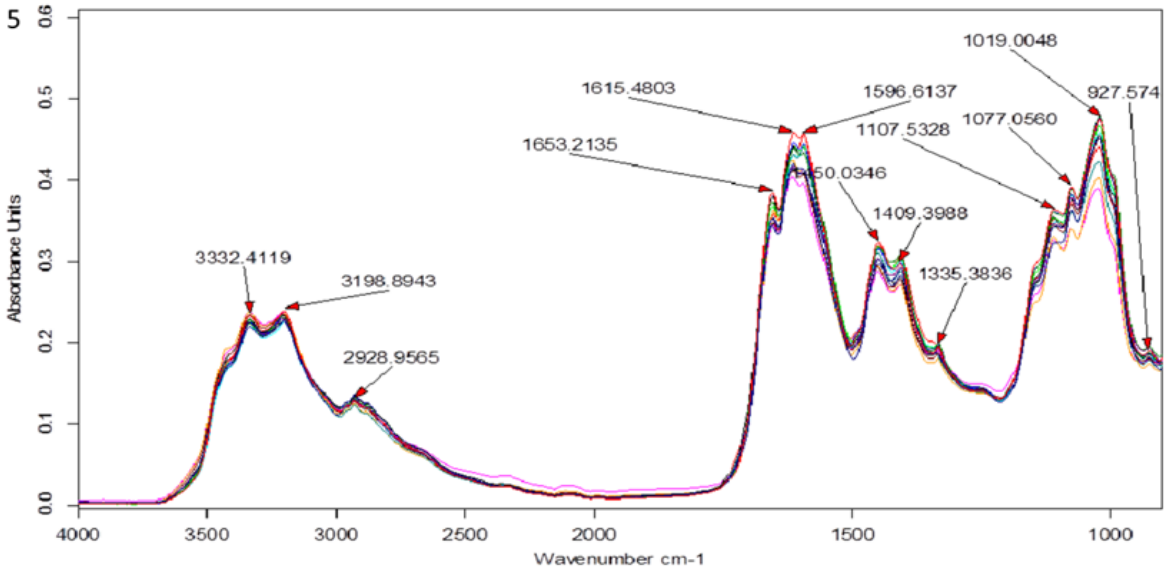


Figure 11 Fourier Transmission InfraRed (FTIR) spectra depicting the reproducibility and stability of the modified Syntho over 24 hours (8 replicates).

3.4. Analytical methodology development

3.4.1. Solid phase extraction

A solid phase extraction protocol was developed and tested as appropriate sample preparation, aimed at improving the chromatography and mass spectrometry detection and quantification and to preserve the performance of the instrument. The protocol followed a previous method for the multi-residue analysis of pharmaceuticals in wastewater (Vergeynst et al., 2015).

3.1.1. The samples were filtered through a 0.7 µm GF/F filter (Whatman), the first 2 ml of sample were discarded to prevent sorption losses (Kiehm and Dressman, 2008). Several cartridges were tested for recovery: OASIS HLB (Waters), StrataX (Phenomenex), and Telos (Kinesis). The following features were common to the three tested cartridges: 200 mg polymeric sorbent and 6 cc (mL) barrel volume.

The recovery of all the compounds was tested as well with several eluents, namely methanol only, methanol with 2% formic acid, and methanol with 5% ammonia. After elution the samples were blown down under nitrogen at 40 ˚C and reconstituted in 1:10 proportions of methanol and water.

The OASIS HLB cartridges with methanol plus 2% formic acid as eluent performed the best, and therefore were adopted in the methodology. The results of the SPE recovery and matrix effect tests for the seven selected APIs and the chosen cartridge are provided in Figure 12.

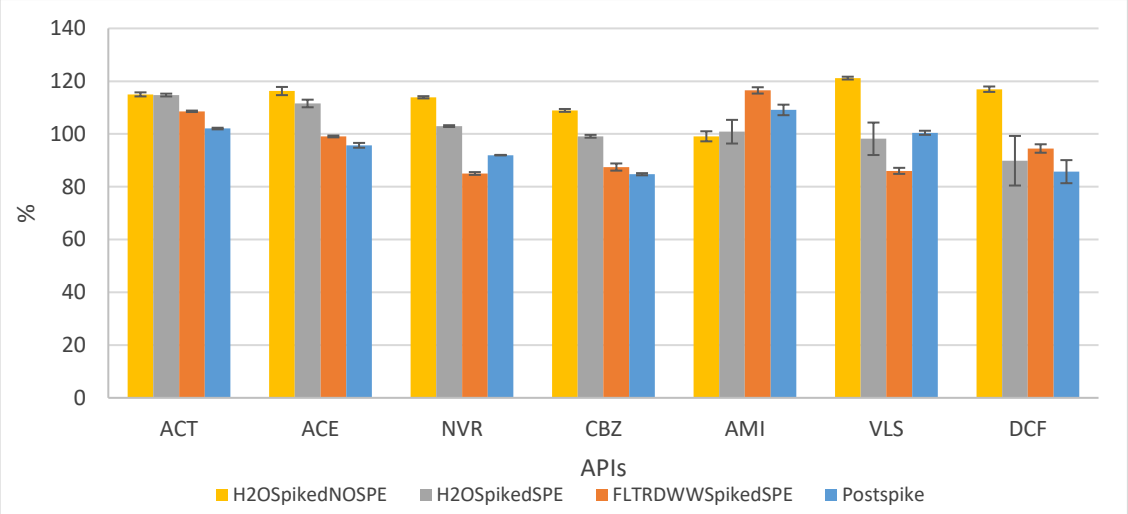


Figure 12 shows the solid phase extraction (SPE) recovery (%) of APIs in ultra-high purity water (UHP) and the matrix effects in filtered synthetic wastewater and reconstituted sample. It is also shown the analysis of UHP with APIs and no SPE. Acetaminophen (ACT); acebutolol (ACE); nevirapine (NVR); carbamazepine (CBZ); amitriptyline (AMI); valsartan (VLS); diclofenac (DCF).

The recovery of all the APIs in UHP was optimal and no significant differences were detected between the analyses of the solid phase extracted APIs and the APIs standards in UHP (Figure 12). The observable matrix effects caused by the filtered synthetic wastewater are likely to result from sorption to the organic matter dissolved in UHP.

3.4.2. Liquid chromatography and mass spectrometry

3.4.2.1. HPLC-MS methodology

The HPLC-MS method development for the analysis of the seven selected APIs was conducted over a period of several months to achieve the best sensitivity and resolution in a single methodology, to be applied throughout the laboratory scale tests. This included the choice of the mass spectrometer settings, e.g. positive or negative

modes, resolution, scan range, etc. and the chromatographic conditions, e.g. column, injection volume, eluents, etc.

Chromatographic separation for the seven APIs was obtained using a Thermo Scientific Dionex U3000 UHPLC system. The chromatography column was an ACE UltraCore 2.5 SuperC18 (100 x 2.1 mm, 2.5 µm) (Serial number A156729; Batch: V14-8055; Catalogue number: CORE-25A-1002U) operating at a temperature of 50°C. During the preliminary screening phase of the methodology development injection volumes between 5 - 20 µL were tested. The injector wash was a solution of water/methanol 1:1% v/v. The tested aqueous eluents were 0.1% formic acid in water (Fluka, LC/MS Chromasolv) and 0.1% ammonia in water, whilst the organic eluents were methanol (Thermo Fisher Scientific, Optima LC/MS) and acetonitrile. The eluent flow rate was set at 500 µL min^{-1}.

After a few tests, the best chromatographic conditions were obtained with a sample injection of 5 µL, and water (0.1% v/v formic acid) and methanol as the mobile phase solvents. The gradient program ran from 95:5 proportion of D:A to 0:100 for a duration of 5.5 minutes. A constant elution of 0:100 was kept for the duration of 2 minutes. The equilibration time was run at the initial conditions (95:5) for 2.5 minutes (Figure 13).

High Resolution Accurate Mass - Mass Spectrometry (HRAM-MS) was performed by means of a Thermo Scientific - Q Exactive Focus - Quadrupole-Orbitrap.

The ionisation source was Heated Electro-Spray Ionisation (HESI), and set as follow: Sheath gas 53 Arb (nitrogen); Auxiliary gas 14 Arb (nitrogen); Sweep gas 3 Arb (nitrogen); Vaporiser temperature 300 °C; Polarity Positive and/or negative ion; Spray voltage (+) 3500/ (-) 2500 V; Capillary temperature 270°C; S-lens RF level 50.

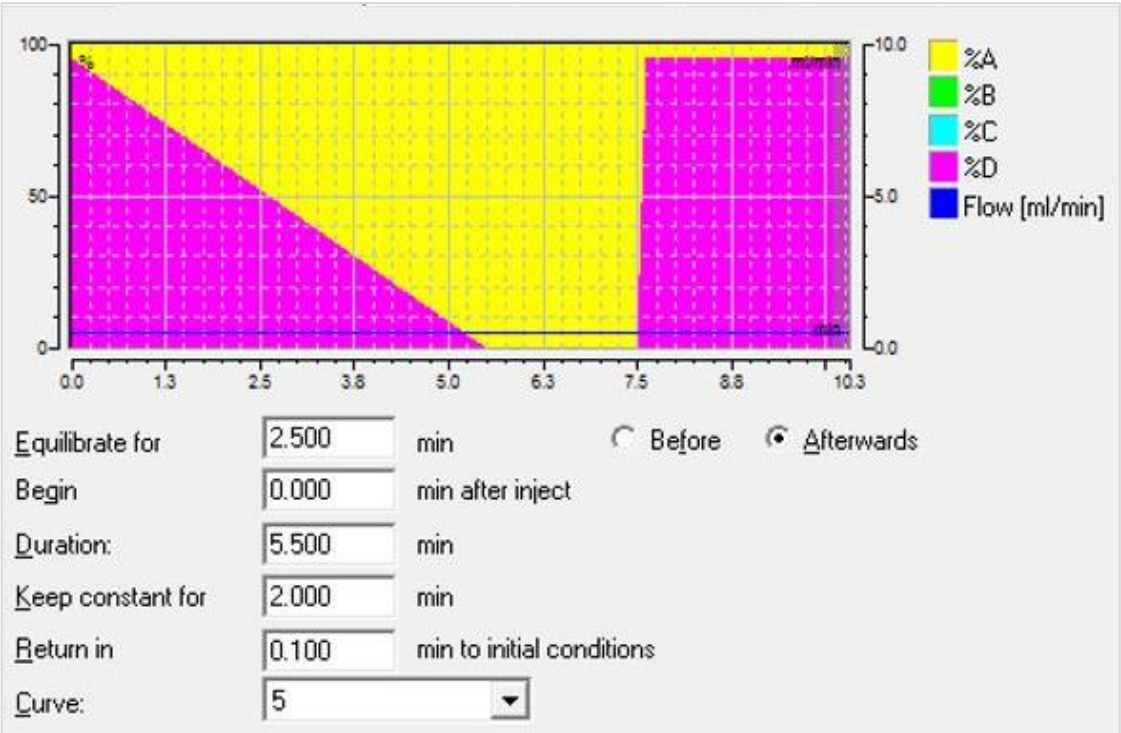


Figure 13 shows the high pressure liquid chromatography (HPLC) gradients conditions from the system software interface (Dionex Chromeleon). %D, ultra-high purity water amended with 0.1% formic acid; %A, methanol.

The mass spectrometer detector settings were as follow: Resolution 17,500 and 35,000 @ m/z 200; Polarity positive or negative ions; Scan range full scan m/z 100 - 1000; AGC target 1.0E6 (automatic gain control); Micro scans 1; Maximum ion time was set as automatic. The mass calibration was performed by external injection of the following mass calibration solutions: auto-calibration using Pierce LTQ Velos ESI positive ion calibration solution (n-butylamine, caffeine, MRFA, and Ultramark 1621, Thermo Fisher Scientific, UK) and Pierce LTQ Velos ESI negative ion calibration solution (sodium dodecyl sulphate, sodium taurocholate and ultramark 1621, Thermo Fisher Scientific, UK).

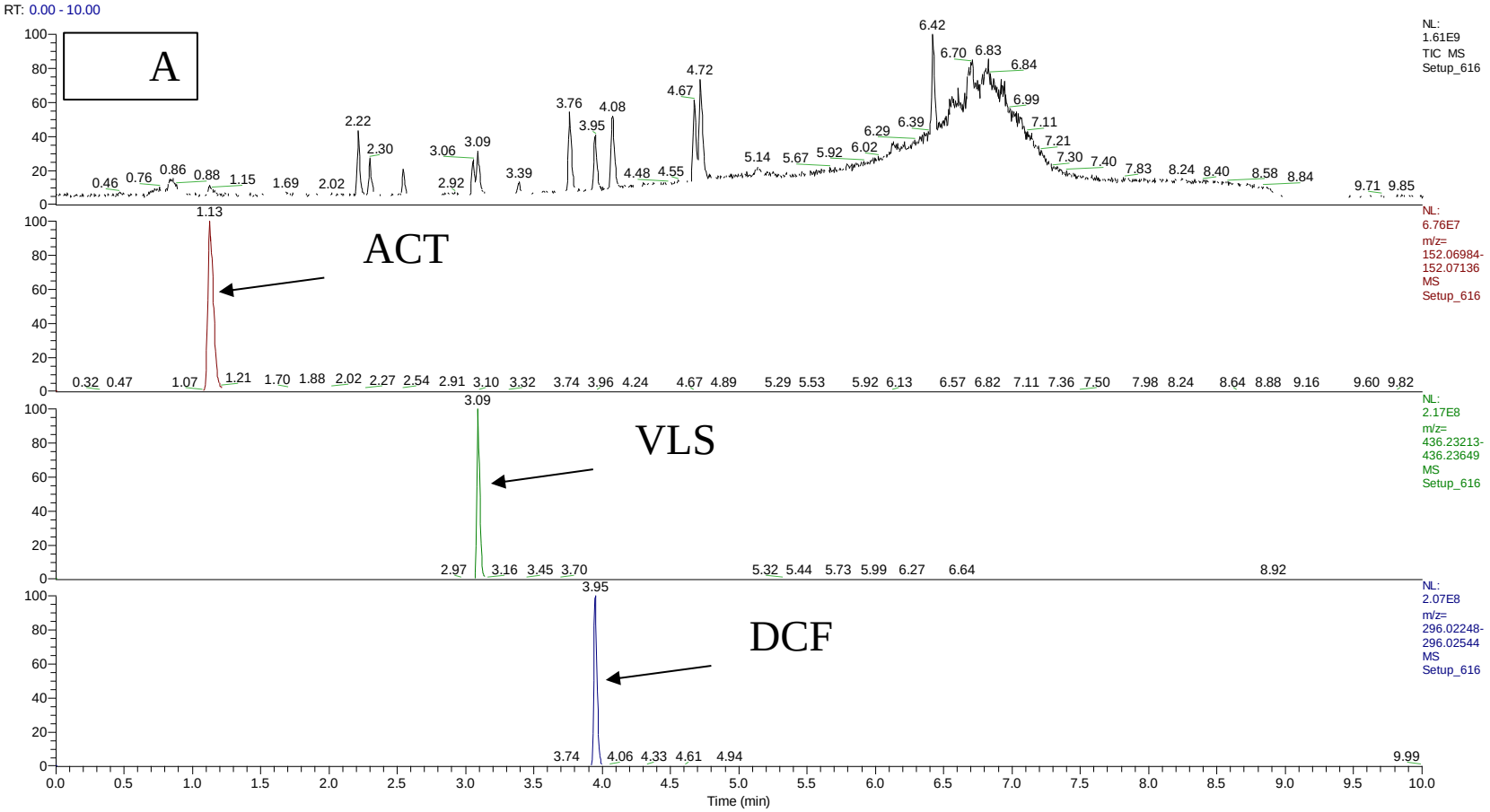
3.4.2.2. Data analysis

The data analysis was performed by means of the Thermo Excalibur software by Thermo, UK. The qualitative and quantitative browsers were respectively used for the chromatogram qualitative analysis and the quantification of analytes in the samples.

The samples were analysed in triplicates and the quality along the run time was checked by quality control (QC) standards and blanks set at regular intervals throughout the run.

A chromatogram is provided in Figure 14 (A and B) to show the peak quality and the retention times of the seven APIs under the methodology chromatographic conditions (acetaminophen (ACT); valsartan (VLS); diclofenac (DCF); nevirapine (NVR); carbamazepine (CBZ); acebutolol (ACE); amitriptyline (AMI)).

The detection and integration of the analyte peak were achieved using the ICIS algorithm set as in Figure 15. The quantification was obtained through calibration curved obtained from external calibration standards spanning eight concentrations, i.e. 0, 0.1, 1, 10, 25, 50, 100 µg L^{-1} (Figure 16).


RT: 0.00 - 10.00
A
NL:
1.61E9
TIC MS
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NL:
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152.07136
MS
Setup_616
VLS
NL:
2.17E8
m/z=
436.23213-
436.23649
MS
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DCF
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2.07E8
m/z=
296.02248-
296.02544
MS
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Time (min)

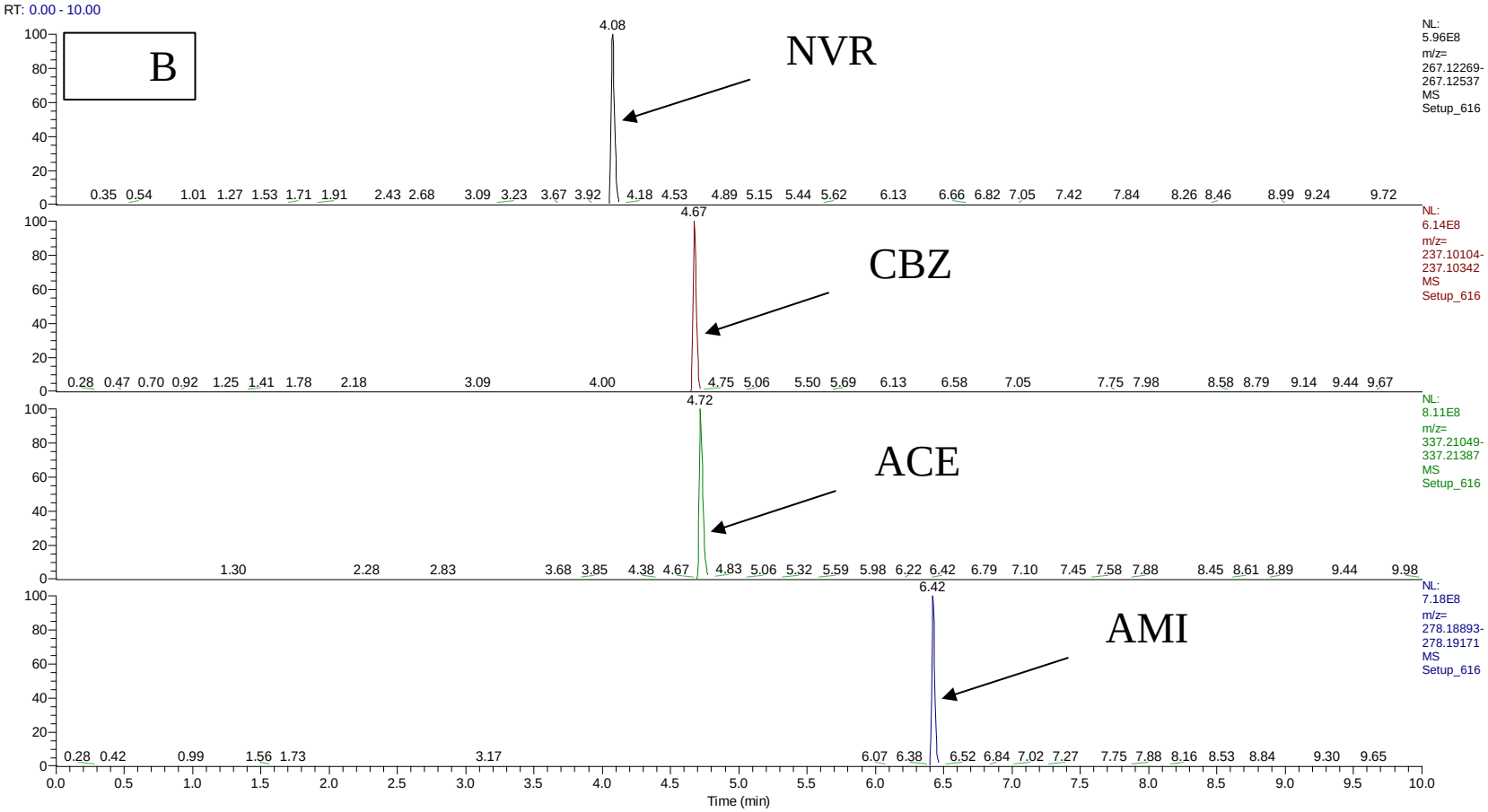

Figure 14 A and B show the peak quality and retention times of the seven APIs selected for this study (Acetaminophen (ACT); valsartan (VLS); diclofenac (DCF); nevirapine (NVR); carbamazepine (CBZ); acebutolol (ACE); amitriptyline (AMI)) under the methodology chromatographic conditions.

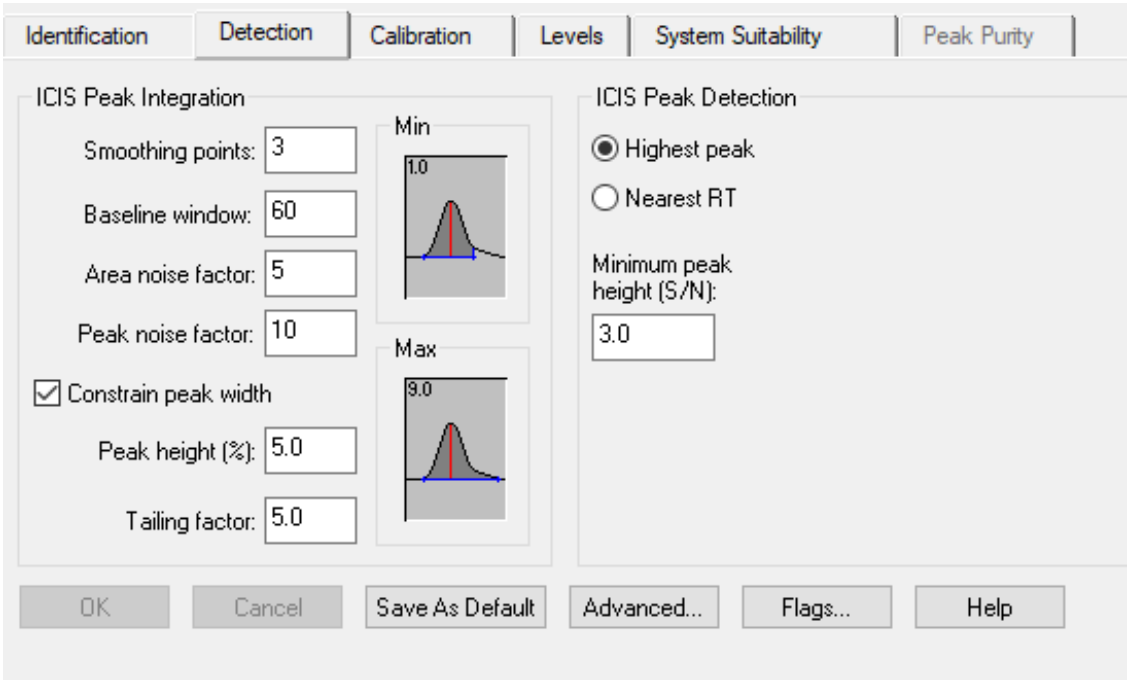

Figure 15 shows the settings for peak detection and integration used in the software Excalibur (Thermo, UK).

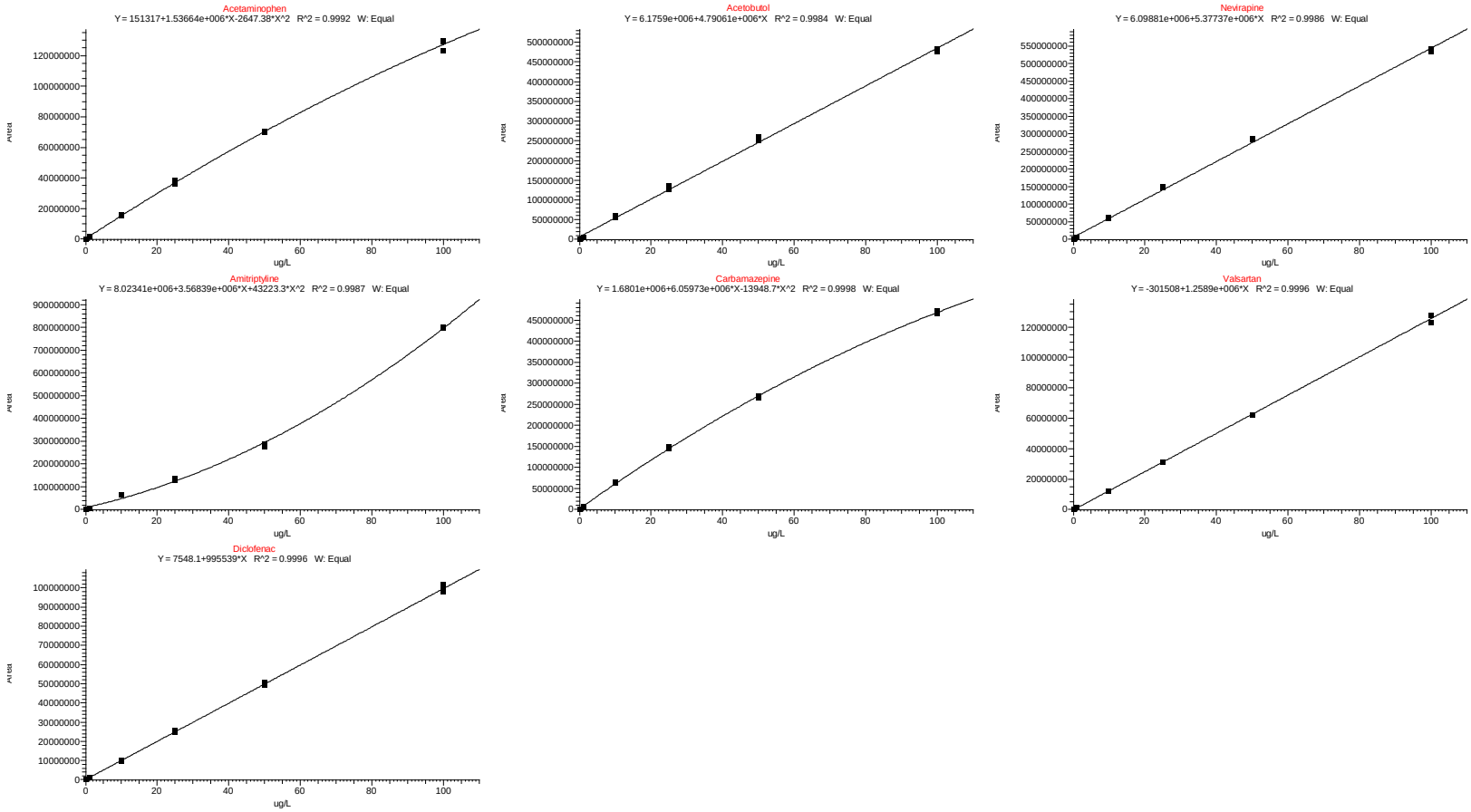

Figure 16 shows the external calibration curves used for the quantification of the APIs.

3.4.2.3. HPLC-MS direct injection methodology

The subset of 55 target APIs (A1) were selected from the list of APIs validated in the methodology of Furlong et al., (2014) and analysed at the Department of Environmental Sciences, University of York, York, United Kingdom.

The determination of APIs in filtered water was achieved by a "direct aqueous injection - high performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS) system methodology", developed and validated by the United States Geological Survey (USGS) agency (Furlong et al., 2014).

Briefly, the method is validated for the determination of the 55 human-use APIs analysed in this work using "a direct-injection" of 100 µL volume of the pre-filtered (0.7 µm mesh glass filter) sample in an HPLC-MS/MS using an electrospray ionization source set in the positive mode. An inline stain-less filter was applied before the column (4.6 mm, 0.2 µm). The APIs were separated using a reversed phase column (Zorbax Eclipse plus-C18 HPLC column, 1.8 µm particle size, 3.0 inner diameter and 100 mm of length) with a gradient of water modified with formic acid/ammonium formate and methanol.

The use of multiple reaction monitoring (MRM) was adopted to enhance the sensitivity and specificity of electrospray HPLC/MS/MS for the qualitative determination of the compounds in the matrix. An internal standard method using stable isotope dilution standards (IDS) of target pharmaceuticals and the pesticide atrazine was

used for quantification (Furlong et al., 2014). The goal of the methodology development was to provide a routine method for the determination of APIs at limit of detection (LOD) and quantification (LOQ) below 50 ng L^{-1} (A2).

3.4.3. Fluorescence spectrophotometry

A fluorescence spectrophotometry methodology was used for the characterization of the synthetic wastewater and for the qualitative evaluation and quantification of protein-like DOM as a wastewater marker in the samples from the Nairobi/Athi river catchment sampling campaign.

The methodology consisted of placing an aliquot of the pre-filtered sample (0.7 µm) in a quartz glass cuvette (Hellma fluorescence cuvette 200-2500 nm spectral range) which was analysed using 3D-fluorescence scan mode on an Hitachi F-4500 fluorescence spectrophotometer. The emission range was comprised between 300 and 500 nm, whilst the excitation range was between 200 and 400 nm. The other parameters are shown in Table 8.

Table 8 Instrument parameters for the 3D-fluorescence scan mode (EX: excitation; EM: emission; WL: wavelength).

Instrument Parameters	
Measurement type:	3-D Scan
Data mode:	Fluorescence
EX Start WL:	200.0 nm
EX End WL:	400.0 nm
EX Sampling Interval:	10.0 nm
EM Start WL:	200.0 nm
EM End WL:	500.0 nm
EM Sampling Interval:	10.0 nm
Scan speed:	2400 nm/min
EX Slit:	5.0 nm
EM Slit:	5.0 nm
PMT Voltage:	950 V
Response:	0.004 s
Corrected spectra:	Off
Shutter control:	Off

The methodology was based on the most commonly studied DOM fractions detected in natural waters, namely humic substances, humic and fulvic acids, and amino-acids of proteins and peptides, and their specific EX/EM peak positions (Hudson et al., 2007)(Figure 17).

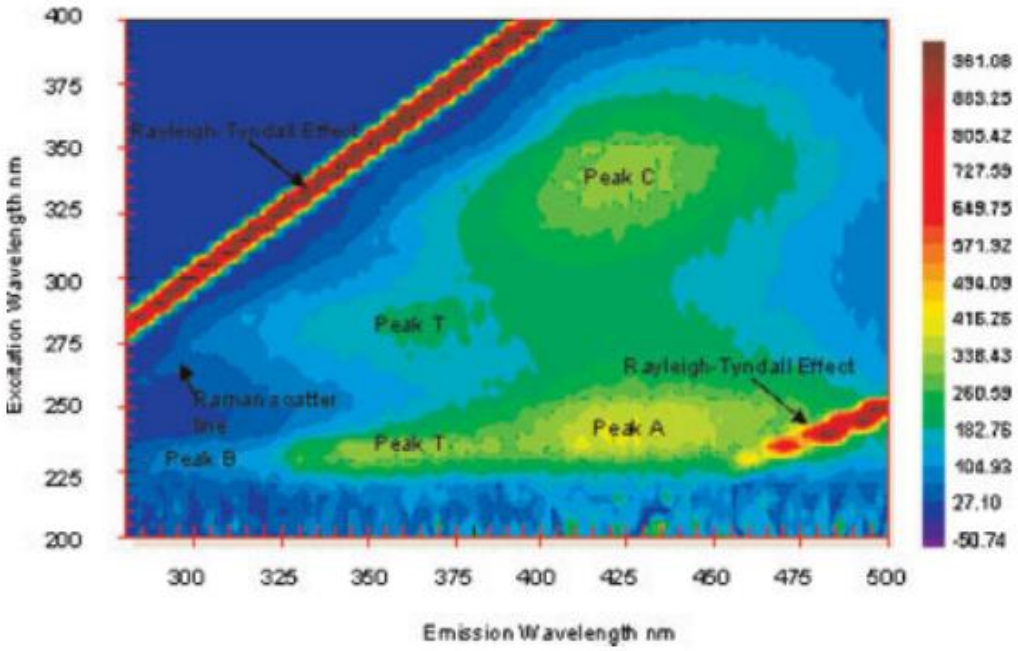

Figure 17 Excitation/Emission Matrix (EEM) showing the common peak positions for humic and fulvic acids (A and C) and protein-like compounds (T). Also, some common features of EEMs are indicated (Hudson et al., 2007).

The protein-like DOM, namely tyrosine and tryptophan-like were quantified by means of external calibration curves (Figure 18 and Figure 19).

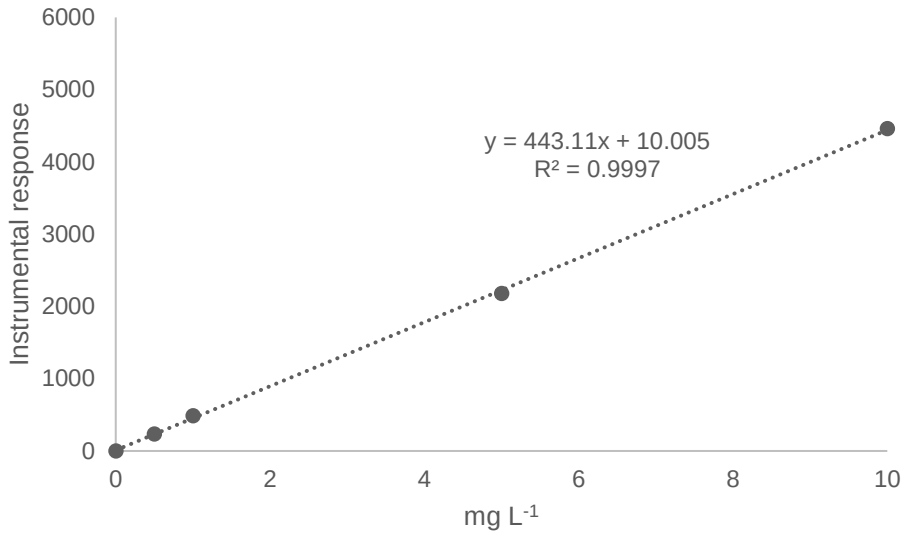

Figure 18 external calibration curve for the quantification of tyrosine-like compounds (EX/EM 230/290).

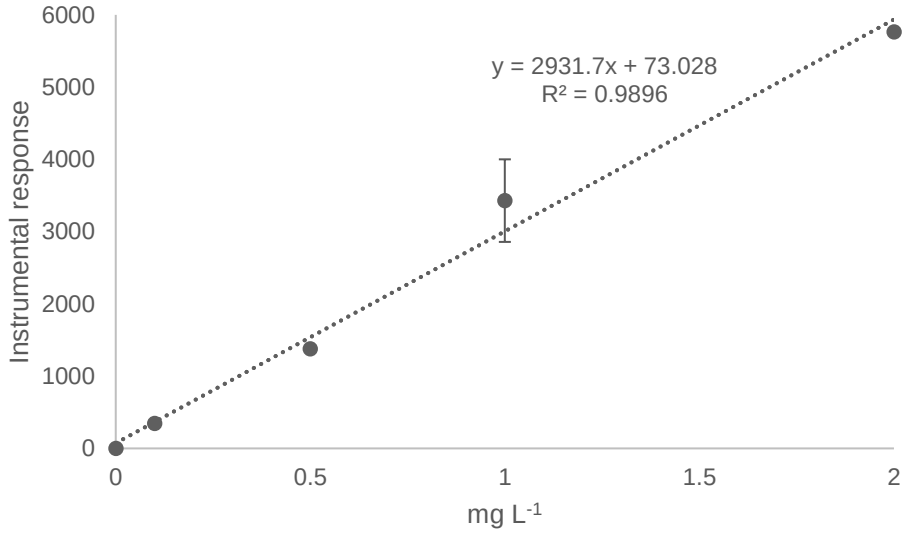

Figure 19 external calibration curve for the quantification of tryptophan-like compounds (EX/EM 230/350).

The peaks of fluorescence in the excitation/emission matrix (EEM) which correspond to tryptophan (230/350) and tyrosine (230/290) were used as a proxy of sewage contamination according to the method proposed by Bagnis et al., (2019), fluorescence spectrometry of the dissolved organic matter (DOM) can be used to characterize the extent of the impact zone. The samples were diluted to a TOC level of 5 mg L^{-1} or less to allow quantification and to minimize the filter effects. The analyses were performed in triplicates using 1 ml of pre-filtered sample in a fluorescence spectrophotometer, Hitachi F-4500. A 3-D scan was performed at a range from 200 to 500 nm for both excitation and emission at a sampling interval of 10 nm and 2400 nm/min of scan speed. A blank of ultra-high purity water (UHP) was subtracted from the samples to eliminate the signal noise from the actual sample. An external calibration curve from tryptophan (Acros Organic) and tyrosine (Sigma) standards was used to quantify the amount of both the tryptophan and tyrosine-like DOM.

3.4.4. Total dissolved organic carbon

The analyses of the total dissolved organic carbon (TDOC) were performed by means of a high-temperature catalytic oxidation (HTCO) coupled to chemilumiscence detection (Badr et al., 2003). The measurements were performed using a Shimadzu TOC-V analyser fitted with an ASI auto-analyser according to the method of Badr et al., (2003). The samples were previously filtered through a 0.7 µm glass filters, diluted 20

times, and acidified to avoid any bacteriological growth and therefore to preserve the samples (10 µL 6 M HCl per 10 ml sample). Subsequently the samples were frozen at -20 °C until the time of analyses.

The samples were defrosted and loaded on the instrument in acid washed and combusted auto-sampler vials. In order to remove dissolved inorganic carbon the samples were sparged using high purity oxygen (8 min at 75 mL min^{-1}). Subsequently, the samples were injected into a combustion column pre-heated at 650 °C and the DOC oxidised to CO_2 in the presence of a platinum coated aluminium oxide catalyst. The CO_2 was detected by means of a non-dispersive infrared detector (NDIRD). The recorded voltage peak measurement was used for quantification of DOC through a series of external calibration standards (Badr et al., 2003).

4. SORPTION OF ACTIVE PHARMACEUTICAL INGREDIENTS IN UNTREATED WASTEWATER EFFLUENT AND EFFECT OF DILUTION IN FRESHWATER: IMPLICATIONS FOR AN "IMPACT ZONE ″ ENVIRONMENTAL RISK ASSESSMENT APPROACH

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Abstract

Evidence of ecotoxicological effects of active pharmaceuticals ingredients (APIs) has increased research into their environmental fate. In low and low-middle income countries (LLMICs) the main source of APIs to surface waters is from discharge of untreated wastewater. Consequently, concentrations of APIs can be relatively high in the "impact zone" downstream of a discharge point. Little is known about the fate of APIs in these impact zones. In this laboratory scale investigation, the effect of successive dilution of synthetic untreated wastewater (dilution factor 1 to 10) on the distribution of APIs was studied. The sorption was consistent with the chemical properties of each compound: charge, lipophilicity, and structure. Dilution increased desorption of the basic and neutral APIs (up to 27.7%) and correlated with their lipophilicity (R^2>0.980); the positive charge was of secondary importance. Anions did not significantly desorb (< 10% loss). Increased concentrations of dissolved organic matter at dilutions of 8 and 10 times that of untreated wastewater coincided with lower dissolved API concentrations. The data showed a clear trend in the desorption process of APIs that may lead to higher exposure risk than anticipated. Therefore, it is suggested that these aspects should be accounted for in the development of dedicated environmental risk assessment approach for APIs in riverine impact zones of LLMICs countries.

4.1. Introduction

The increasing consumption and production of active pharmaceutical ingredients (APIs) in low and low-middle income countries (LLMICs) is growing environmental concern owing to the awareness of possible ecotoxicological effects (Kookana et al., 2014). This is related to the diffused practice of direct discharge of untreated wastewater (DUW), the main source of APIs to the environment, which creates a heavily polluted area downstream from the discharge point, named the "impact zone" (A.I.S.E./CESIO, 1995; Finnegan et al., 2009; Kookana et al., 2014; Malik et al., 2015; Nansubuga et al., 2016; Thebo et al., 2017).

Little is known about the environmental fate of APIs in the "impact zone" created by the DUW. Nevertheless, a few available measured environmental concentrations (MECs) of APIs in impact zones of LLMICs show higher concentrations than for high-income countries with developed wastewater treatment infrastructure (Madikizela et al., 2017). For instance, in the Nairobi River basin, Kenya, APIs were detected at concentrations ranging from ng L^{-1} to 160 µg L^{-1} (K'oreje et al., 2016, 2012; Ngumba et al., 2016), in Nigeria were reported individual concentrations above 50 µg L^{-1} (Olatunde et al., 2014), and again, in South Africa were detected concentrations of atenolol and ibuprofen up to 30 and 85 µg L^{-1} respectively (Agunbiade and Moodley, 2016, 2014; Matongo et al., 2015), and antiretroviral were quantified at concentrations up to hundreds of ng L^{-1} (Wood et al., 2015). Pharmaceutical factories wastewater was thought to be the source of APIs concentrations up to mg L^{-1} in Pakistan (Ashfaq et al., 2017) and India (Larsson, 2014); and in tropical Asia, sulphonamides antibiotics in surface waters were found to be at higher concentrations than in high-income countries (Shimizu et al., 2013). In one reported case, the environmental risk assessment showed a potential for risk, and

pharmaceutical manufactory wastewater contribution was deemed as important, as also evidenced by other investigations (Ashfaq et al., 2017; Larsson, 2014; Ngumba et al., 2016). Although API manufacturing sites would be expected to be identified as high risk, it should also be noted that in high income countries direct discharge of untreated wastewater from such factories is illegal. The reported data for LLMIC countries therefore highlights the environmental concerns and need for carefully considered risk assessment.

As demonstrated above, globally there are common occurrences of API concentrations in "impact zones" which exceed 0.01 µg L^{-1} for any individual compound. Under the existing risk assessment process, if predicted, such a PEC would trigger Phase II of the environmental risk assessment (ERA) (EMA, 2006), which consists of a two-step tiered protocol to evaluation of the risk. Tier A is an initial environmental fate and effects analysis that, if resulting in a risk, should be followed by Tier B, an extended environmental fate and effects analysis (EMA, 2006). The latter is a refinement of the predicted environmental concentration (PEC) in the surface water using a distribution coefficient, which considers the moiety adsorbed to sewage sorbents as being retained in the wastewater treatment sludge (OECD, 2000). Equation 3 is used for PEC refinement in tier B of the ERA:

Equation 3

$$PEC_{surface\ water} = \frac{Elocal_{water} * F_{stp\ water}}{WASTEW_{inhab} * CAPACITY_{stp} * FACTOR * DILUTION}$$

Where $PEC_{surface\ water}$ is the output of the local surface water concentration (µg l^{-1}); $Elocal_{water}$ is the local emission to wastewater of the relevant residue (µg L^{-1}); $F_{stp\ water}$ is the fraction of emission directed to wastewater (µg L^{-1}); $WASTEW_{inhab}$ is the amount of wastewater per inhabitant per day (L d^{-1}); $CAPACITY_{stp}$ is the capacity of the local wastewater treatment plant (l); *FACTOR* accounts for adsorption to suspended matter; and *DILUTION* is the DF, with a default value of 10 (EMA, 2006).

Where untreated wastewater is discharged there is little or no retention of sludge, the entire crude sewage is input to the "impact zone" scenario. Consequently, the sorbents loaded with APIs are discharged and diluted with the receiving freshwater, and possible redistribution processes might cause imprecise calculation of PECs and the associated risk quotient.

Engineering protocols recommended a ratio of river flow to untreated wastewater flow of 40 (DF) (Keller et al., 2014) to allow dilution and dispersion of pollution. A DF of 10, assuming previous wastewater treatment, is used as the default value for environmental risk assessment (EMA, 2006; European Comission Joint Research Centre, 2003).

Although risk assessments are inherently designed to be conservative, reported data suggests that this level of dilution may not always occur. In at least 14 countries worldwide, the local predicted DF median observations show a value below 10, the majority being in North Africa and the Middle East, with Belgium as the only European country (Keller et al., 2014). The number increases to 53 countries worldwide if data of observations falling in the 5 and 25 percentiles are considered (Keller et al., 2014) . The APIs sorption processes to wastewater sorbents control the exposure to biota (Agunbiade

and Moodley, 2016; Carmosini and Lee, 2009; Hernandez-Ruiz et al., 2012b; Hudson et al., 2007; Lahti and Oikari, 2011; OECD, 2000; Peng et al., 2014; Svahn and Bjorklund, 2015; Wang et al., 2016; Zhou et al., 2007), and since DUW occurs at dilutions that can cause significant desorption of APIs (Hajj-Mohamad et al., 2017; S. F. Yang et al., 2011) such exposure might be underestimated with simple dilution calculations.

The aim of this study was to assess the partitioning of APIs to wastewater sorbents, and to quantify the potential dilution-induced desorption in receiving freshwaters using a standardised synthetic untreated wastewater diluted across a range of DFs. This approach was designed to assess the effect of the major constituents present in untreated wastewater (particularly the presence of high concentrations of organic carbon, potentially capable of 'stabilising' APIs in the dissolved phase) on the environmental fate of APIs. Outcomes of the study could then be used to inform the development of an improved exposure assessment approach for a range of contaminants in the impact zone generated by the DUW in freshwaters.

4.2.Experimental approach

Triplicate SW incubations (500 mL) were spiked with APIs each at a concentration of 100 μg L^{-1}. This concentration was chosen for the following reasons: (i) it represented levels that have been observed in impact zones (Azuma et al., 2017; Kookana et al., 2014; Madikizela et al., 2017); (ii) levels were not high enough to bias any physico-chemical effects which might occur in the impact zone; (iii) concentrations were high enough to allow accurate and precise determination using the applied analytical technique in the dissolved phase after equilibration (particularly for the strongly adsorbing APIs). Samples were continually stirred and progressively diluted using UHP (MilliQ, deionised

water resistivity of at least 18.2 MΩ•cm at 25 °C). A pH of 7.5 was chosen as representative of the environmental and wastewater matrix. Sample blanks and controls were included (Figure 20). The flasks were wrapped in aluminium foil to avoid exposure to light. The dilution distribution dynamics were tested over a range of ten dilution factors (DF): 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 4, 8, 10. The DFs were based on the progressive achievement of DF 10, which is the environmental risk assessment default assumption (EMA, 2006; Keller et al., 2014). After each dilution, the sample was left for 24 hours to reach equilibrium before sampling, which was a conservative time estimate (Conrad et al., 2006; S. F. Yang et al., 2011).

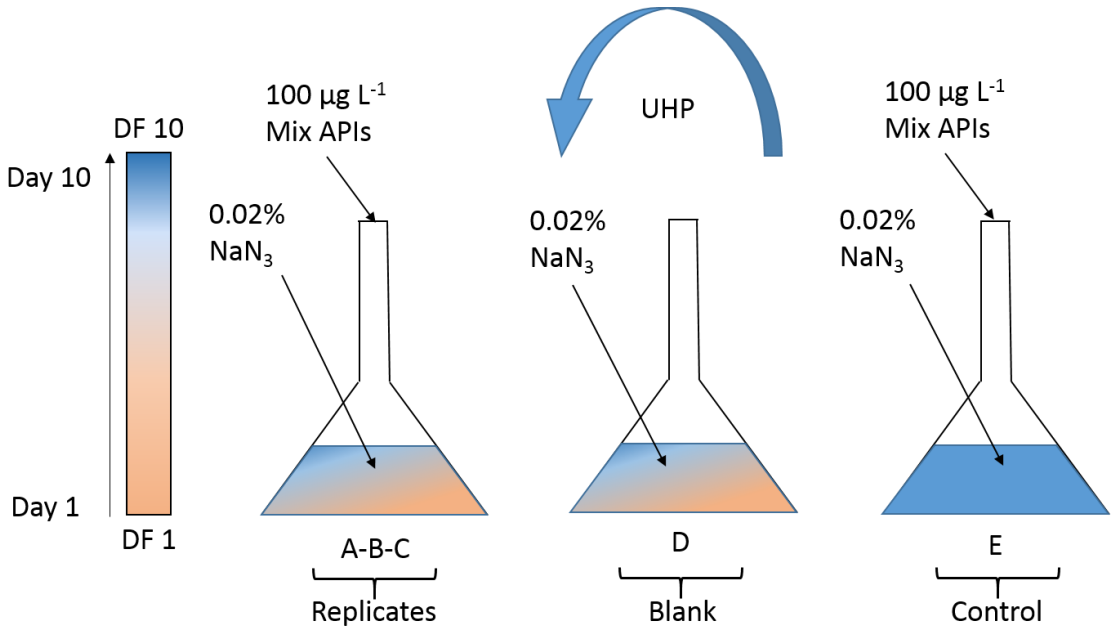

Figure 20 Experimental design of the dilution experiment. A-B-C were sample replicates; D was the blank and E was the control where APIs were added to buffered and sterilized (NaN3) ultra-high pure water (UHP). Each batch was progressively diluted with UHP from the dilution factor (DF) 1 to 10 (1, 1.2, 1.4, 1.6, 1.8, 2, 4, 6, 8, 10) along a period of 10 days.

4.3.Experimental approach

4.3.1. Calculations

4.3.1.1. Determination of K_d values

The environmental fate of a contaminant is largely determined by its sorption behaviour. The extent of sorption is expressed as the distribution coefficient, K_d, normally determined by the particulate : dissolved ratio at equilibrium (Franco and Trapp, 2008). In this study the concentration in solids refers to sorption to the bulk sorbents of untreated wastewater, including colloids and DOM, and therefore hereafter named as concentration in sorbents (C_s) whilst the concentration in water (C_w) to the freely available fraction.

Therefore, the $K_{d\ exp.}$ is obtained from the ratio of the compound concentration in the sorbent phase (C_s) and in the aqueous phase (C_w) (Equation 4):

Equation 4

$$K_d = \frac{C_s}{C_w}$$

The distribution coefficient was calculated at each DF. The modelled distribution coefficient values ($K_{d\ Mod.}$) were also calculated for comparison to the experimental ones. The pH dependent octanol-water distribution coefficient (D_{ow}), which accounts for compound dissociation, dependent on the pK_a, was calculated for each compound functionality, according to Equation 5Equation 6Equation 7 (neutral, acidic and basic, respectively):

Equation 5

$$\log D_{owN} = logK_{ow}$$

Equation 6

$$logD_{owA} = logK_{ow} + log\frac{1}{1+10^{pH-pKa}}$$

Equation 7

$$logD_{owB} = logK_{ow} + log\frac{1}{1+10^{pKa-pH}}$$

Where log D_{ow} is the distribution coefficient octanol-water (log K_{ow}) adjusted to the dissociation of the compound at a given pH; pKa is the dissociation constant of the compound (Lin et al., 2010).

Log D_{ow} was related to K_d using Equation 8 (Lin et al., 2010):

Equation 8

$$\log K_{d\ Mod.} = 0.74 \times \log D_{ow} + 0.15$$

4.3.1.2. Variation from theoretical concentration (% VTC)

In order to evaluate extent of desorption for each API, the theoretical concentration was calculated at each DF, including undiluted sample (DF1), and subtracted from

experimental data. The results were recalculated as the percentage of variation from the theoretical concentration (%VTC) for normalisation, as shown in Equation 9:

Equation 9

$$\%\boldsymbol{VTC} = (\boldsymbol{C_{exp}} - \boldsymbol{C_{th}/C_{DF1}}) \times \mathbf{100}$$

Where C_{exp} is the API experimental concentration in water, C_{th} is the API theoretical concentration in water and C_{DF1} was the API concentration in water at DF 1.

4.4.Results and discussion

4.4.1. Sorption

The API distribution in undiluted samples (DF 1) is presented in Figure 21 as a percentage of the compound remaining in solution and ordered per log K_{ow}.

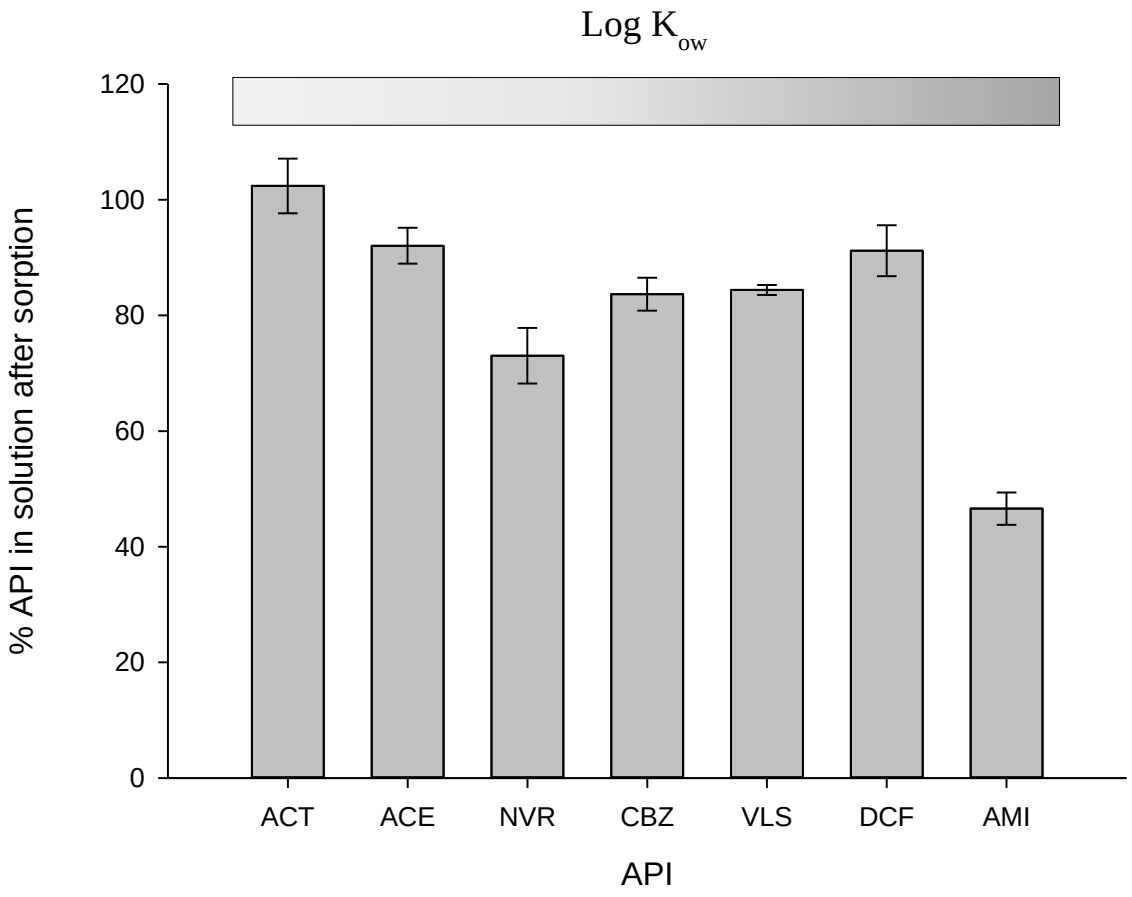

Figure 21 Percentage (%) of APIs in solution after spiking at no dilution (DF 1); the compounds are ordered per increase of API log Kow, as indicated in the top bar. The error bars show the standard deviation (ACT, acetaminophen; ACE, acebutolol; NVR, nevirapine; CBZ, carbamazepine; VSL, valsartan; DCF, diclofenac; AMI, amitriptyline).

These data were used as the initial concentration for the calculation of the theoretical concentration after dilution and the log Kd (Table 9).

Table 9 Measured concentration of APIs in undiluted solution (DF1) and experimental solid-water distribution coefficient values.

DF 1	ACT	ACE	NVR	CBZ	VLS	DCF	AMI
µg L^{-1}	102.39	92.05	73.05	83.68	84.42	91.20	46.58

Log K_d [L kg^{-1}]	-1.70	2.27	2.90	2.62	2.60	2.32	3.39

* ACT, acetaminophen; ACE, acebutolol; NVR, nevirapine; CBZ, carbamazepine; VSL, valsartan; DCF, diclofenac; AMI, amitriptyline.

At the experimental pH of 7.5, the acidic and basic APIs are calculated to be fully ionised, and the compounds defined as neutral, ACT (pKa 9.38), NVR (pKa 2.8) and CBZ (pKa 13.2), may be considered fully unionised. The measured sorption behaviour was consistent with the chemical properties of each compound: charge, lipophilicity, and structure, and in accord with previous studies (Jelic et al., 2011; Silva et al., 2011; Verlicchi et al., 2012).

The low log K_{ow} (0.3) of the neutral ACT predicts little sorption, which agrees with previously published studies (Li et al., 2015; Lin et al., 2010; Martínez-Hernández et al., 2014). The neutral CBZ and NVR, log K_{ow} of 2.7 and 2.5 respectively, show a similar sorption trend. Sorption was greatest for AMI, consistent with its high lipophilicity (log K_{ow} 4.9) and the influence of the positive charge. In fact, the lipophilic interactions are reported to be most important, whilst the charge on the ionised functional group exercises a secondary control on the distribution processes (Franco and Trapp, 2008; Githinji et al., 2011; Martínez-Hernández et al., 2014; Silva et al., 2011). The low sorption of ACE was supported by its log K_{ow} (1.7), which confirmed the secondary impact of the positive charge in determining the sorption behaviour. DCF and VLS, however, adsorbed less strongly than expected per their relatively high log K_{ow} (3.6 and 4.3, respectively). This was likely due to the degree of repulsion of the negative charge on both the API and sorbent competing with lipophilic attraction (Delle Site, 2001; Paul et al., 2014).

The log K_d obtained at DF1 were compared with values available in the literature (Table 10) (Al-Khazrajy and Boxall, 2016; Bai et al., 2008; Hernandez-Ruiz et al., 2012b; Lahti and Oikari, 2011; Li et al., 2015; Lin et al., 2010; Loffler et al., 2005; Maoz and Chefetz, 2010; Martínez-Hernández et al., 2014; Maskaoui et al., 2007; Maskaoui and Zhou, 2010; Stein et al., 2008; Svahn and Bjorklund, 2015; Yamamoto et al., 2009; Zhou and Broodbank, 2014). The data show the importance of the sorbent quality (i.e. protein-like or humic-like organic matter) in determining the extent of API sorption. Wastewater is mainly composed of proteinaceous material which binds organic contaminants more weakly than humic-like substances typical of freshwater (Hernandez-Ruiz et al., 2012b; Peng et al., 2014; Wang et al., 2016). The characterization of the synthetic wastewater used during this study confirmed the predominance of proteinaceous components (Figure 9) and the $K_{d\ exp.}$ were consistent with its comparative binding strength. In fact, the log K_d of -1.70 L kg^{-1} for ACT was in the range of values obtained for suspended solids (SS) (-2.2 and 0.5) in a simulated sewage system (Hajj-Mohamad et al., 2017). Also, the log K_d for CBZ (2.62 L kg^{-1}) obtained in this study corresponded to the value reported by Maoz and Chefetz (Maoz and Chefetz, 2010) for DOM extracted from bulk sewage sludge (2.64 L kg^{-1}), and in the range obtained by Lahti and Oikari (Lahti and Oikari, 2011) for sediments from wastewater effluent (2.00-3.42 L kg-1) (Table 4). CBZ sorption to humic-like substances revealed a much larger log K_d in contrast of up to 6.66 L kg^{-1} (Table 4). The proteinaceous composition of the SW could explain the lack of ACE sorption despite the positive charge, consistent with the range (log K_d of 0.5 - 1.0 L kg^{-1}) obtained by Lahti and Oikari (Lahti and Oikari, 2011) for particulate matter derived from wastewater treatment works effluent, considerably less than 3.28 L kg^{-1}, obtained by Lin et al. for freshwaters, typically characterized by the presence of humic-like substances (Lin et al., 2010). However, the repulsion of negative charges on the dissociated acidic

compounds is more important in sorption processes than the sorbent quality. This was shown by the log $K_{d\ exp.}$ 2.13 L kg^{-1} for DCF obtained for synthetic humic-like SS by Ra et al. (2008) that was close to the value of 2.32 L kg^{1} obtained in this study (Table 9).

4.4.2. Trend of dissolved concentration of APIs as a function of dilution

The variation in concentration of the dissolved APIs with dilution is shown in Figure 22. Desorption is expressed as the percentage of variation from the theoretical concentration (%VTC) against DF. The extent of the deviation, as a dilution effect, varied between compounds, determined by the relative influence of compound functionality and lipophilicity.

Maximum deviation was measured at low DF, namely DF 2, whilst at higher DF the concentrations of APIs are similar to the theoretical values. The highest %VTC occurred for AMI (27.7%), followed by CBZ (12.4%), NVR (11.8%), ACE (7.7%), DCF (4.2%), ACT (1.3%), and VSL (-3.2%).

Figure 22 shows the behaviour of the compounds separated by functionality. The compound ACT showed no variation from the theoretical concentration at each dilution factor (Figure 22A), which was expected as sorption was insignificant (Figure 22). ACT (pK_a 9.38) was neutral at the experimental pH so functionality would not have influenced sorption. The low log K_{ow} (0.3 L Kg^{-1}) indicates negligible lipophilicity, consistent with the low retention shown by the wastewater sorbents. As such, ACT behaved conservatively at each DF. The neutral compounds NVR and CBZ show a similar trend of deviation from predicted concentration (+10 %VTC at DF2). The two APIs were both neutral at the experimental pH and their log K_{ow} values are similar (2.50 and 2.67 L Kg^{-1}, respectively), which explains the similar trend, and highlights the role of lipophilicity

in controlling the sorption of APIs to and from the wastewater sorbents. CBZ and NVR have similar molecular structures that could be the cause of the notable persistence of the former (Andreozzi et al., 2004), and, if true also for the latter, would help explain the ubiquitous presence of NVR in impact zones (K'oreje et al., 2016, 2012; Ngumba et al., 2016).

Figure 22B shows the trend in the deviation from the theoretical concentration for the basic compounds AMI and ACE. AMI shows the largest %VTC (27.7%) amongst the compounds investigated, which is concomitant with the largest log K_{ow} value (4.9). ACE is a cation at the experimental pH, but the lipophilicity (log K_{ow} 1.7) appeared to be the only physico-chemical parameter affecting desorption.

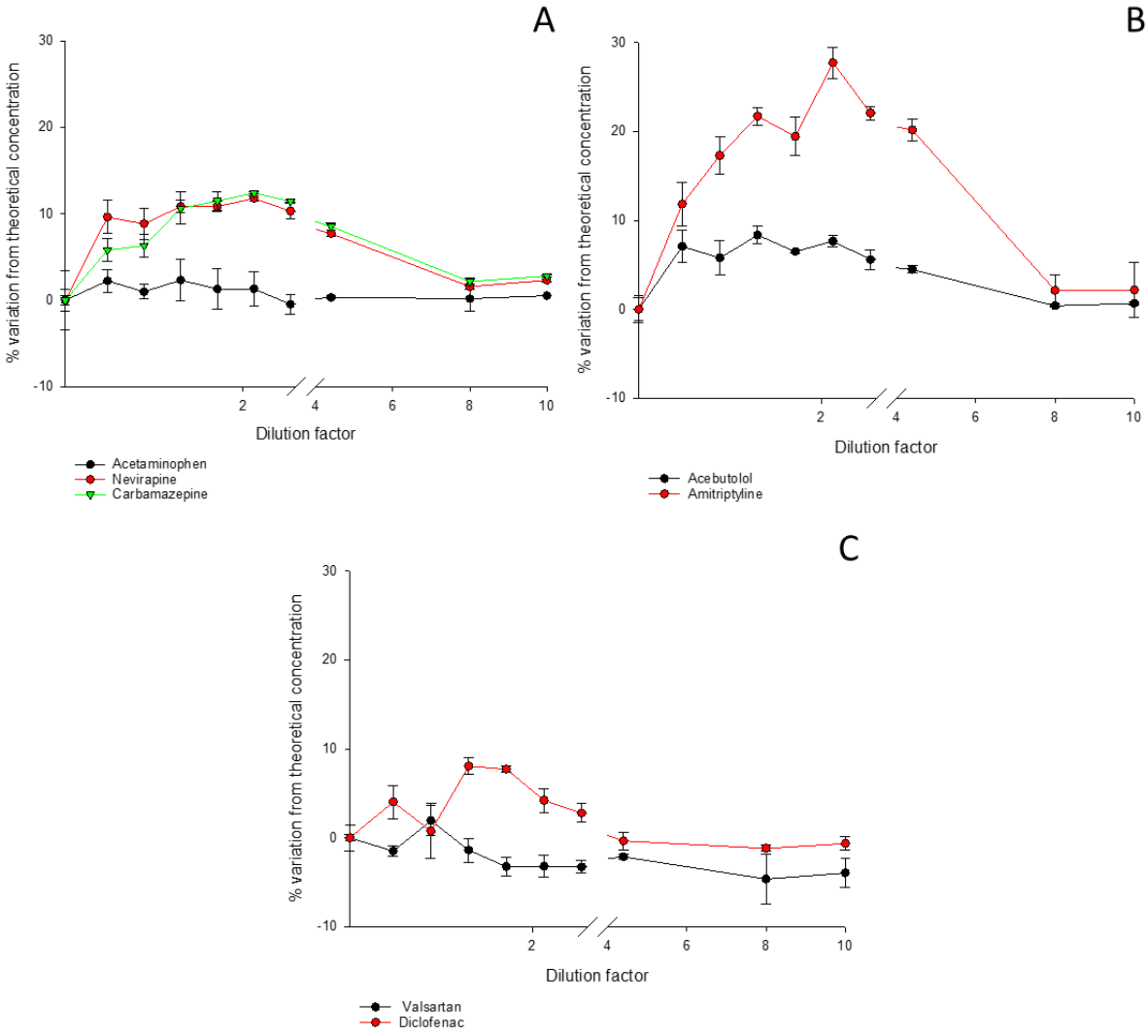

Figure 22 Percentage of variation from theoretical concentration of A: neutral compounds acetaminophen (ACT), Nevirapine (NVR), and carbamazepine (CBZ); B: basic compounds Acebutolol (ACE) and Amitriptyline (AMI); C: acidic compounds Valsartan (VLS) and Diclofenac (DCF); at DF from 1 to 10.

Figure 22C shows the behaviour of the acidic compounds VLS and DCF. As previously discussed, these acidic compounds showed little sorption, despite the large log K_{ow}, likely due to repulsion between the negative charge on the compound and the negative net charge of the organic matter sorbents (Refaey et al., 2017). Also, little desorption was measured for DCF (10%VTC) and none for VLS. The former behaviour was likely determined by strong binding of electrical forces involving charge transfer

(~40 kJ mol^{-1}), which regards the moiety of negative charged compounds that once adsorbed would be unlikely reversible (Martínez-Hernández et al., 2014).

Lipophilicity was the main parameter determining the behaviour of the neutral and cationic APIs, whilst the negative charge on the anionic APIs strongly interfered with the sorption/desorption processes. This trend is shown in Figure 23A, which depicts the relationships between log K_{ow} and the %VTC of neutral and cationic APIs, on the right of the black line, and acidic compounds, on the left. Figure 23B shows the correlation of the %VTC and the log K_{ow} of the neutral and positively charged compounds with the coefficient of determination (R^2) greater than 0.950 in 7 of the 9 DFs.

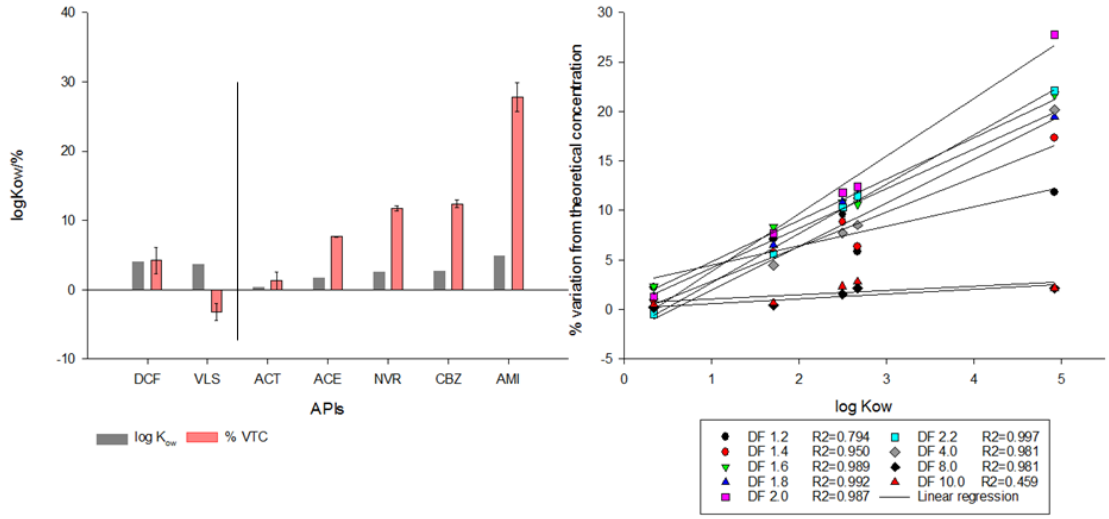

Figure 23 A shows the relationships between the log Kow of neutral and positively charged APIs on the right of the black line at the percentage of variation from theoretical concentration (%VTC) of 2, and the lack of relationship of the acidic compounds, on left side; B shows the correlation of the neutral and positively charged compounds versus dilution.

4.4.3. Modelled versus experimental K_d

The log K_d values for the APIs were obtained from experimental data (log $K_{d\ exp.}$) and a theoretical model (log $K_{d\ mod.}$) (Lin et al., 2010). Additionally, literature solid-water distribution coefficients (log $K_{d\ lit.}$) were collected (Table 4), and the upper and lower values added to Table 2 for comparison.

Although the log $K_{d\ mod.}$ at DF 1 did not exactly match the experimental values, the data were within the range of reported values from the literature, which demonstrated the validity of the model (Table 10). The calculated log K_d for AMI was closest to the experimental value (DF 1), but did not correspond to the reported range of values from the literature. However, the log $K_{d\ lit.}$ values for AMI originated from a single study and related to distribution to sediments, whilst the ranges for other compounds related to more relevant sorbents, namely DOM, colloids and suspended solids. As previously discussed, the sorbent type and quality strongly affect distribution processes and, therefore, the K_d values.

A general increase of log K_d occurred at DF 8 and 10 for all APIs, especially NVR, CBZ, VLS and AMI, where this was up to one order of magnitude (Table 10). These increases were related with increased concentration of dissolved organic carbon (DOC) at the DF of 8 and 10, as depicted by the plot of theoretical and experimental DOC in Figure 24. As the DOC concentration is a representative measure of the concentration of DOM, it follows that the dissolution of organic matter from particulate organic matter (POM) increments the cation exchange capacity because of the increase in specific surface area, and therefore sorption. Therefore, the decrease of API concentration at the DF of 8 and 10 is likely due to an interplay of dilution and additional sorption to the

proportionally increased DOM, with respect to the expected concentration from the theoretical calculation.

Table 10 Modelled (Log Kd mod.), reported values from the literature (log Kd lit.), and experimental (Log Kd exp.) distribution coefficient values for the APIs investigated in this study, including DFs (Al-Khazrajy and Boxall, 2016; Bai et al., 2008; Hernandez-Ruiz et al., 2012; Lahti and Oikari, 2011; Li et al., 2015; Lin et al., 2010; Loffler et al., 2005; Maoz and Chefetz, 2010; Martínez-Hernández et al., 2014; Maskaoui et al., 2007; Maskaoui and Zhou, 2010; Stein et al., 2008; Svahn and Bjorklund, 2015; Yamamoto et al., 2009; Zhou and Broodbank, 2014) (See Table S3 for the list of values and respective references).

Log K_d [L/kg]	**DF**	**ACT**	**ACE**	**NVR**	**CBZ**	**VLS**	**DCF**	**AMI**
Log $K_{d\ mod.}$	/	0.40	1.42	2.00	2.13	2.85	3.15	3.79
Log $K_{d\ lit.}$	/	-0.3 - 2.4	0.5 - 3.3	n. a.	-1.5 - 6.7	n. a.	0.9 - 6.9	0.9 - 2.4
Log $K_{d\ exp.}$	**1.0**	-1.70	2.27	2.90	2.62	2.60	2.32	3.39
	1.2	-2.12	0.48	2.78	2.49	2.74	2.08	3.37
	1.4	-2.06	0.94	2.82	2.47	2.68	2.42	3.35
	1.6	-2.32	2.21	2.76	1.77	2.88	2.06	3.32
	1.8	-2.27	2.09	2.75	1.78	3.02	2.21	3.36
	2.0	-2.34	2.44	2.65	2.33	3.08	1.61	3.21
	2.2	-1.80	2.24	2.73	2.40	3.14	2.19	3.32
	4.4	-2.54	2.94	2.32	3.10	3.47	3.04	3.12
	8.0	-2.80	2.96	3.57	2.51	4.18	3.56	4.16
	10.0	-3.19	2.61	3.39	3.16	4.32	3.57	4.22

n. a.: not available

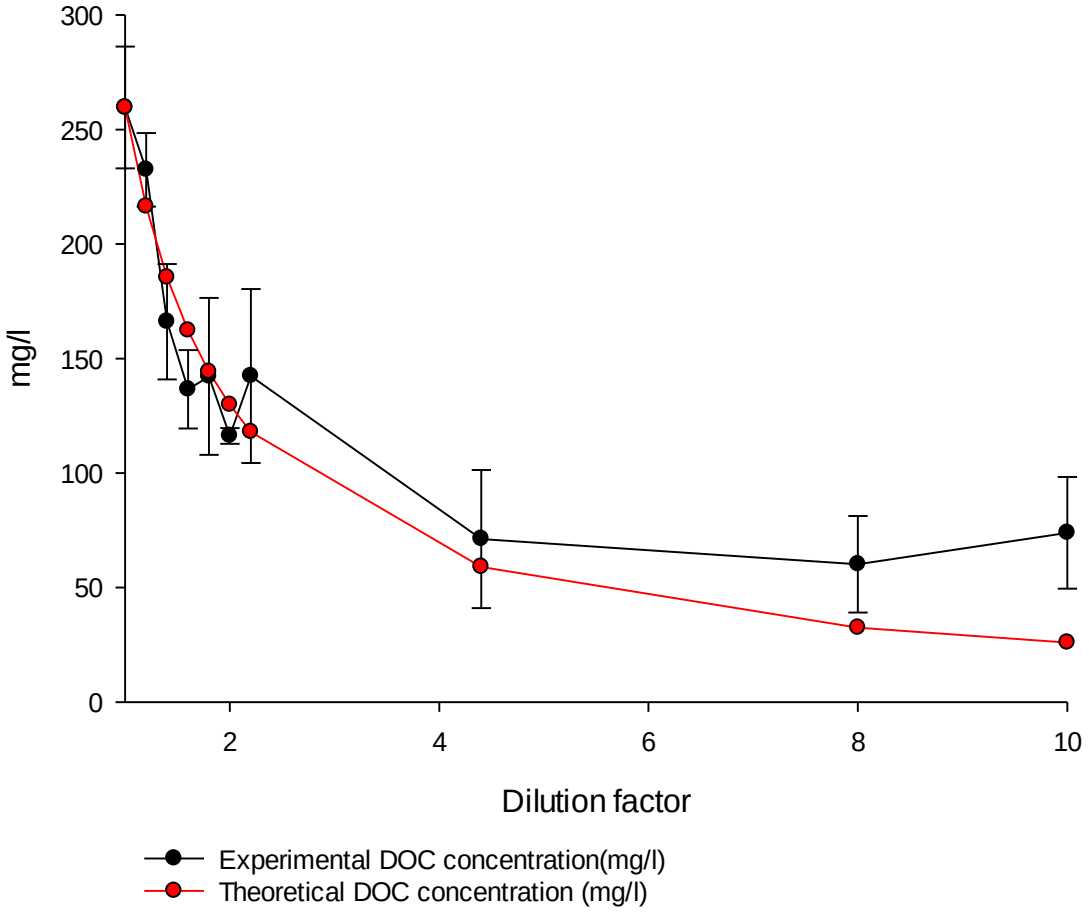

Figure 24 Theoretical and experimental dissolved organic carbon (DOC) concentrations recorded at each DF; and the increase from theoretical concentration at DF 8 and 10.

4.4.4. Implication of API desorption within the impact zone for ERA

ERA guidelines do not include a protocol for evaluating ecological risk posed by the direct discharge of API-containing untreated wastewater (EMA, 2006). Although from a human health and environment perspective such practices should not occur, the fact is that across LLMIC such discharges are widespread. Phase 1 of the ERA guideline is aimed at estimating exposure within the aquatic environment only. It does not consider the route of administration, API form, metabolism and excretion. If the PEC is calculated

above 0.01 µg l^{-1}, then a phase 2 analysis, which includes the generation of environmental fate and effect data, should be performed. However, in the phase 2 tier B environmental fate analysis, the PEC calculation considers the distribution of APIs to the sewage sludge accordingly to the experimental log K_{oc}, defined as the log K_d value normalized to organic content in sewage sludge as from the OECD 106 protocol (OECD, 2000).

Equation 3 may not be applicable to discharges of poorly or untreated wastewater where wastewater treatment is limited or does not occur. In fact, as from the obtained evidence, highly lipophilic neutral or positively charged APIs desorb more readily with dilution (Figure 23), and omitting desorption could lead to potential underestimation of APIs PEC. Municipal and industrial wastewater are considered the primary source of APIs to the environment, while poor or absent wastewater treatment is widespread globally (Malik et al., 2015). This study has identified clear trends in API environmental cycling during wastewater dilution which are not addressed in current APIs environmental risk assessment legislation, and which could have consequences for the estimation of precise environmental concentrations.

4.5. Conclusions

Inadequate wastewater treatment and consequent direct discharge of untreated wastewater to surface waters is a global problem. This study presents data on the sorption of APIs to untreated wastewater sorbents, and their deviation from theoretical concentrations during dilution in freshwaters, for the evaluation of exposure concentrations, using APIs representative of LLMICs.

The measured sorption behaviour was consistent with the chemical properties of each compound: charge, lipophilicity, and structure. ACT was not adsorbed because of

its low lipophilicity and lack of charge, while the behaviour of NVR and CBZ was similar, consistent with the proximity of their log K_{ow} values and chemical structure. The behaviour of the basic compounds, AMI and ACE, indicated that primary control of sorption was lipophilicity with a secondary role for the positively-charged functional group. In contrast, sorption of the acidic compounds, DCF and VLS, was low due to repulsion between the negatively-charged compound and the similar net charge on the sorbent surface sites. The measured log K_d values were consistent with reported values for the types of sorbent studied.

Dilution caused significant positive deviation from theoretical concentrations of the neutral and basic APIs at low dilution factors, and showed a high correlation to the lipophilicity, with the positive charge playing a secondary role. The negatively-charged compounds did not show significant desorption (i.e. 0 % loss for VLS and < 10 % for DCF). This behaviour was attributed to irreversible binding of the negatively-charged functional group to positively-charged sites on the sorbent. In addition to dilution, the concomitant increase in DOM concentration at the higher DF (i.e. 8 and 10) appeared to result in further sorption of APIs.

As a conclusive reflection, the possibility of de-conjugation of conjugates as metabolites could be summed up to the mechanistic desorption magnitude described in the results.

This study has identified clear trends in API environmental cycling during wastewater dilution which are not addressed in current APIs environmental risk assessment legislation, and which could have consequences for the estimation of precise environmental concentrations.

5. IMPACT OF THE WASTEWATER-MIXING ZONE ON ATTENUATION OF PHARMACEUTICALS IN NATURAL WATERS: IMPLICATIONS FOR AN IMPACT ZONE INCLUSIVE ENVIRONMENTAL RISK ASSESSMENT

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Abstract

The direct discharge of untreated wastewater has been identified as an important source of environmental contamination by active pharmaceutical ingredients and other 'down-the-drain' chemicals in developing countries. It necessitates the development of an environmental risk assessment approach for the resulting impact zone. This study was designed to investigate the impact of low level of dilution (<10) on the natural attenuation processes of distribution and degradation within the impact zone. Dilution of the untreated wastewater resulted in increased desorption and corresponding environmental concentrations. The presence/absence of the microbial population in the batches affected the degree of sorption depending on the compound charge (i.e. positive or negative), highlighting an experimental technical bias. The degradation half-lives of acebutolol and diclofenac increased with increasing dilution and resulted in higher environmental persistence. The modelling of the biochemical oxygen demand (BOD) allowed an estimate of the temporal end boundary of the impact zone to be predicted as 24 h. Therefore, it was concluded that most of the investigated compounds would persist beyond the end of the impact zone as defined by the return to environmental BOD concentrations. It is proposed that, within environmental risk assessment protocols, the impact zone should be considered as a semi-natural wastewater treatment area in such a way to allow the estimate of environmental concentrations of pharmaceuticals beyond its end.

5.1. Introduction

Wastewater management is an acute challenge in low and low-middle income countries (LLMICs) where increased volumes of wastewater generated by rapid urbanisation have not been matched by upgrades of sewerage infrastructures resulting in direct discharge of untreated wastewater (DDUW) to surface waters (Malik et al., 2015; Nansubuga et al., 2016). This results in serious pollution of a waterway to such a downstream distance that a combination of dilution and biogeochemical processes such as degradation, volatilisation and sorption render the anthropogenic discharges non-toxic. This area downstream of the DDUW has been defined as an "impact zone", beginning at the DDUW entry point and ending where easily measurable determinants such as biochemical oxygen demand (BOD), ammonia, and dissolved oxygen levels, critical to ecological health, have returned to environmental background concentrations (A.I.S.E./CESIO, 1995; Bagnis et al., 2018a). Whilst DDUW are known to contain high nutrient concentrations and BOD, the presence of emerging contaminants such as active pharmaceuticals ingredients (APIs) is of increasing concern (Keller et al., 2014). This applies particularly to LLMICs where from a pharmaceutical point of view, LLMICs are also experiencing an increase in consumption of medicines, as well as relocation of pharmaceutical production plants to these regions (Kookana et al., 2014).

The impact zone of DDUW has been linked to higher environmental concentrations of APIs than in high income countries with more advanced wastewater treatment plants (Kookana et al., 2014; Malik et al., 2015) and API concentrations up to mg L^{-1} have been detected in impact zones of LLMICs from Africa to Asia (Ashfaq et al., 2017; Madikizela et al., 2017; Ngumba et al., 2016; Shimizu et al., 2013).

To regulate any discharge effectively, it is necessary to be able to define impact zones in order to minimise their extent and plan future wastewater treatment requirements. Furthermore it is necessary to fully understand the fate of chemicals within an impact zone as they are unlikely to behave the same as BOD or ammonia, often used to define such zones. Furthermore, current environmental risk assessment (ERA) guidance for APIs has been developed assuming that sewage is subject to treatment prior to discharge (EMA, 2006), and thus, given that the DDUW does not comply with this assumption, the environmental conditions in the impact zone require a dedicated ERA approach (Bagnis et al., 2018a). This can only be achieved by understanding the mechanisms of natural attenuation that APIs undergo, such as sorption and biodegradation, and the resulting degree of exposure within the impact zone boundaries and beyond.

Additionally, an important mechanism related to the natural attenuation of chemicals in wastewater is dilution (Keller et al., 2014). Many environmental risk assessment and management strategies are overly reliant on the paradigm that dilution reduces a chemical's concentration such that it positively contributes to the reduction of environmental risk in a directly proportional way. In the current ERA protocol, the default ratio of river flow to treated wastewater is set to 10 (EMA, 2006; European Comission Joint Research Centre, 2003). However, despite the conservative nature of the ERA protocol, this dilution factor is overestimated in at least 53 countries, many of which are LLMICs severely affected by DDUW (Keller et al., 2014). Such an assumption may therefore be resulting in under estimates of downstream concentrations of chemicals and given the fact that chemicals are not actually transformed by dilution, this premise is often criticized (Van Breukelen, 2007).

A highly important factor often overlooked are the questions as to what the impacts of dilution are on other key processes, such as sorption and biodegradation, which are currently poorly understood but are critical to the derivation of a scientifically robust risk assessment or for use within the development of water quality models (Bagnis et al., 2018a; Hajj-Mohamad et al., 2017).

The practicalities of measuring APIs in impact zones of LLMICs with substantial inputs of untreated wastewater are highly challenging. The number of environmental variables are high and sample collection, preservation and analysis is often not possible locally. Therefore, it is necessary to develop and adopt laboratory simulations which provide the opportunity to control important variables such as wastewater quality and characteristics, dilution and API concentration and thus provides the ability to generate vitally important fate data upon which to base future risk assessment approaches.

This study focuses on the fate and behaviour of APIs within a simulated impact zone through the recreation of conditions of dissolved organic carbon – DOC (considered a surrogate of BOD), suspended solids and microbiological inoculums on a laboratory scale. Additionally, it investigates the effects of dilution on the main natural attenuation processes controlling the concentration of APIs in the impact zone and beyond, i.e. distribution and biodegradation. Given that there is the potential for some APIs to persist beyond the impact zone defined by parameters such as BOD, for example, owing to either high concentrations discharged or being more recalcitrant than the observed BOD, it is essential to be able to quantify the extent as part of any risk assessment procedure. The overarching aim is to contribute to the development of an approach of environmental risk assessment for APIs within and beyond the impact zone generated by DDUW. Further contributing to the novelty of this research is the development of a simple model based

on simulated BOD concentrations (derived from experimentally derived DOC values) to predict API concentrations outside of the BOD derived impact zone, leading to a draft protocol for the assessment of the environmental risk posed by APIs in LLMICs with limited or poor wastewater treatment.

5.2. Materials and methods

All glassware and plasticware were cleaned prior to use (2 % v/v Decon, ≥ 24 h; 10 % v/v HCl, ≥ 24 h) and rinsed with ultra-high purity water (UHP) with resistivity above 18.2 MΩ cm (Merck Millipore).

5.2.1. Active pharmaceutical ingredients

The APIs chosen for this study were the neutral acetaminophen (ACT), carbamazepine (CBZ), and nevirapine (NVR), the acidic diclofenac (DCF) and valsartan (VLS), and the basic acebutolol (ACE) and amitriptyline (AMI). These compounds represent APIs with different structure and functionalities (neutral, acidic and basic) which are commonly consumed in LLMICs (Bagnis et al., 2018a). The chemicals were purchased at the highest purity available, either from Sigma-Aldrich (acebutolol hydrochloride, amitriptyline hydrochloride, nevirapine, valsartan, and acetaminophen) or Fisher Scientific (carbamazepine and diclofenac sodium salt).

5.2.2. Synthetic wastewater

Wastewater composition varies considerably according to both time and location (Tchobanoglous et al., 2003), presenting challenges in its standardisation for experiments (Boeije et al., 1999). Therefore, a surrogate of synthetic wastewater is needed to achieve experimental reproducibility as well as to ensure there are no background API

concentrations present. Also, in order to perform an ERA for chemicals, some form of standardization is needed to facilitate inter- and intra-lab replication. Therefore, a synthetic medium is a logical starting point for such complex studies (Bagnis et al., 2018a). The experimental design was informed by OECD standards tests for chemicals in the environment, which are also standardized in some respect, and was optimised by a through critical evaluation of synthetic wastewaters reported in the literature (Bagnis et al., 2018a; O'Flaherty et al., 2013). Thus, a synthetic wastewater (SW) was prepared to achieve controlled and reproducible experimental conditions (Boeije et al., 1999). In this study, sodium azide (NaN_3) was added to sterilized batches, whilst inoculum (5 mL; consisting of fresh wastewater influent collected at the local wastewater treatment plant of South West Water, Plymouth, England, UK) was added to all other batches (OECD, 1992). The batches containing the inoculum were pre-conditioned for 3 days before the experiments began (OECD, 1992).

5.2.3. Experimental approach

Dilution factors (DF) of 1, 2, 4 and 10 for untreated SW were prepared in triplicate and a control (500 mL) produced for each DF (Figure 25).

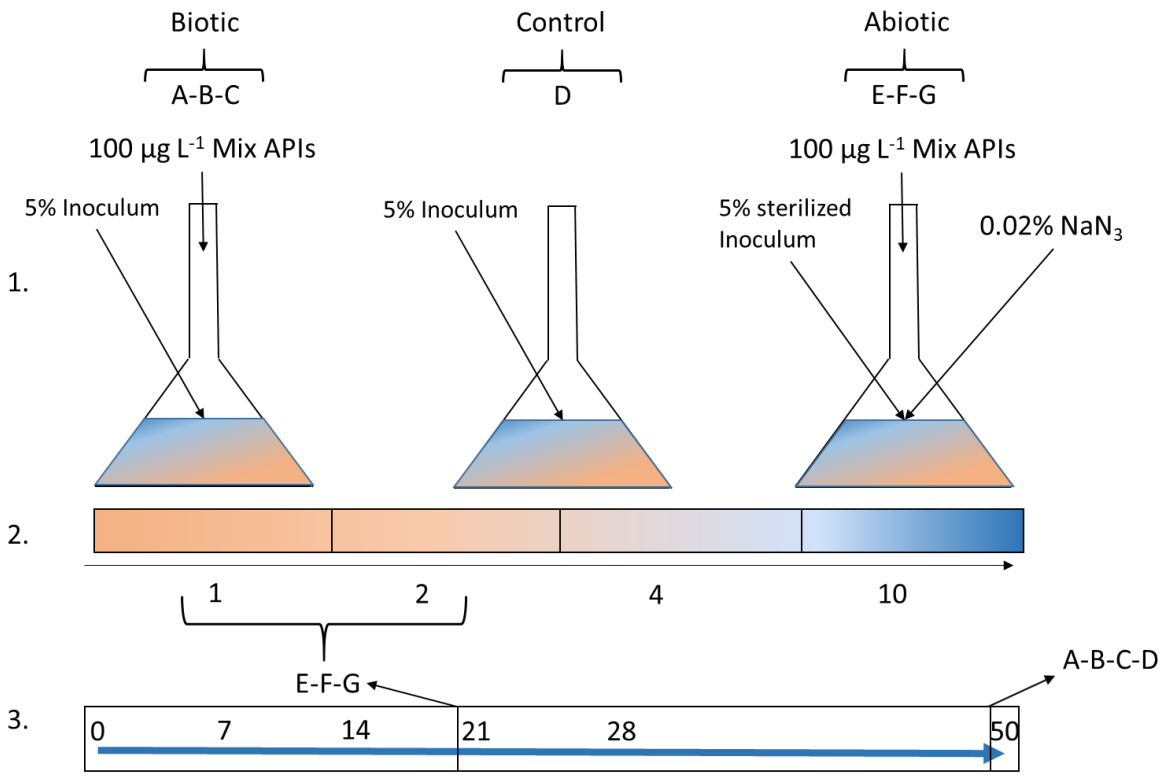

Figure 25 Experimental design aimed at measuring the effect of synthetic wastewater dilution on the natural attenuation of the active pharmaceutical ingredients (APIs); I. the biotic batch in three replicates A, B, and C spiked with 100 µg L-1 of each API and 5 ml of inoculum; D, the control batch added with 5 ml of inoculum; the abiotic batches E, F, and G spiked with 100 µg L-1 of each API, 5 ml of sterilized inoculum, and 0.02% NaN3;II. the dilution factors (DF) of 1, 2, 4 and 10; only 1 and 2 applied to the abiotic batch; III. the sampling schedule over the experimental duration of 50 days.

The SW was buffered at pH 7.5, which is typical for both wastewater and receiving waters. Inoculum was added to each replicate and left for 72 h before addition of 7 APIs, each to a final concentration of 100 µg L^{-1} as a representative concentration for impact zones of LLIMCs (K'oreje et al., 2016, 2012; Madikizela et al., 2017; Ngumba et al., 2016). The flasks were wrapped in aluminium foil to avoid photodegradation and continuously stirred. The temperature was 21 °C during the experimental period. After an equilibration time of approximately 4 hours, batches were diluted with UHP at a DF

of 1, 2, 4, and 10. Samples were collected at several time points over 50 days for API and DOC determination. An identical abiotic experiment was performed for DF 1 and 2 only, and run for 20 days to determine the sorption component only and/or hydrolysis in order to quantify loss through biodegradation (Figure 25). This second batch was kept sterile by adding sodium azide (0.02%).

5.2.4. Analytical procedures

Samples were collected in 15 mL centrifuge tubes (Fisher Scientific) and centrifuged for 10 minutes at 4000 rpm. The supernatant was filtered through 0.7 µm GF/F filters (Whatman) in order to allow for solid phase extraction (SPE) and preconcentration without interference from suspended solids present at high levels in the simulated untreated wastewater. For analysis of DOC, an aliquot was diluted 20 times (owing to the high levels present) acidified (HCl, 6 M) and frozen at -20 °C for preservation prior to analysis (Badr et al., 2003).

Concentrations of APIs were measured according to Bagnis et al. (2018). Briefly, aqueous samples were passed through a SPE cartridge for sample clean-up and to optimise analyte resolution, mass detection and quantification. The SPE cartridges (OASIS HLB, 200 mg polymeric sorbent; 6 mL barrel volume; Waters, UK) were activated with 5 mL of methanol (Thermo Fisher Scientific, Optima LC/MS) and 5 mL of UHP. A 5 mL sample aliquot was then loaded onto the cartridge at a flow rate of 3 mL min^{-1}, followed by 1 mL of UHP. The APIs were eluted with 5 mL of methanol amended with formic acid (2 %). The eluent was evaporated to dryness under a gentle stream of nitrogen gas then reconstituted with 1 ml of methanol : UHP at a 1 : 10 ratio.

Chromatographic separations were measured by high performance liquid chromatography (HPLC) coupled with a high-resolution mass spectrometry (HR-MS) orbitrap-based system (Thermo Scientific), using a reversed phase column (XBridge BEH C18 2.5 µm 2.1 x 50 mm Column XP, Waters) maintained at 50 ^{o}C. A gradient elution programme was employed, starting at 100 % UHP amended with 0.1 % formic acid, progressing to 100 % methanol after 5.5 minutes. The parameters used for high resolution mass spectrometric detection are described in Bagnis et al. (2018).

The dissolved organic carbon (DOC) analyses were performed using high-temperature catalytic oxidation (TOC-5000A - Shimadzu) according to the method of Badr et al. (2003).

5.2.5. Calculations

Biodegradation rates were assumed to follow first-order kinetics (Schwarzenbach et al., 2003). The 50 % dissipation time (DT_{50}) was modelled as a surrogate measure of the biodegradation rate according to Equation 1:

$$DT_{50} = \frac{0.5IC - I}{K} \quad (1)$$

Where I is the intercept and IC is the initial concentration after dilution in µg L^{-1} and K is the slope of the equation of the line obtained from the linear trend line of the biodegradation starting at the first sample taken after dilution; the dissipation time is expressed in days.

BOD was not directly determined owing to practicalities of sample size and time constraints. However, reliable datasets are available that provide a strong correlation

between measured DOC and BOD for untreated municipal wastewater (Comber et al., 2018; Kwak et al., 2013). Based on data from over 100 UK wastewater treatment works crude sewage the following relationship was derived from a DOC vs BOD correlation of $R^2 = 0.86$ (Comber et al., 2018):

Equation 10

$$BOD = \frac{DOC+9.9851}{0.2876}$$

5.3.Results and discussion

5.3.1. Effects of dilution on natural attenuation

Three out of the seven APIs investigated, namely ACE, DCF and ACT showed effective biodegradation within the simulated impact zone. The biodegradation rate of ACE and DCF was strongly affected by the dilution, showing an overall increase of persistence (longer half-life) at increased dilution. This inverse correlation is well described by the half-life dissipation time (DT_{50}) of DCF and ACE (Table 1).

Table 11 The 50 % dissipation time (DT_{50}), in days, of the active pharmaceutical ingredients (APIs) acetobutol (ACE) and diclofenac (DCF) caused by biodegradation only, calculated after each applied dilution factor (DF), namely 1, 2, 4 and 10; mean of each experimental replicate (3) and standard deviation (STD).

DT_{50} (Days)	DF1		DF2		DF4		DF10	
	Mean	**STD**	**Mean**	**STD**	**Mean**	**STD**	**Mean**	**STD**
DCF	38.55	17.98	20.93	2.10	36.36	18.07	116.22	80.40
ACE	20.16	1.56	25.63	0.28	26.69	6.54	37.69	6.26

ACE biodegraded over fifteen days then reached a pseudo-plateau with a much slower rate of biodegradation (Figure 26A). The removal rate at no dilution (DF 1) was approximately 78 % after 14 days. This was similar to Lin et al. (2010) who ascribed the disappearance of the compound to sorption processes. However, based on the tests carried out here and examining the data for the abiotic tests here (Figure 26B), it appears that the sorption of ACE was much less significant than biodegradation (Lin et al., 2010). Lin et al. (2010) suggested that their results were most likely due to experimental artefacts which might explain such difference between the two studies. Nonetheless, the progressive dilution from DF 1 to 10 reduced the original concentration of the API, but also the biodegradation rate (Figure26A) and therefore the removal of ACE from the solution. In this case, the progressive increase of dilution concurrently reduced the degrading microbial biomass present in the system, which might explain the trend. This observation is consistent with previously reported studies for other compounds in continuous input studies (Landa et al., 1994).

In the case of DCF the increasing dilution specifically affected its biodegradation profile. In fact, at zero dilution (DF1) the biodegradation curve of DCF showed immediate removal after addition (Figure 26C), whilst a progressive increase in the lag phase prior to biodegradation was observed at DF 2 and 4, lasting respectively 14 and 28 days. At a dilution factor of 10 the DCF concentration remained constant after dilution, for the duration of the whole test (50 days), thus rendering the compound highly persistent. It is worth noting that, although DCF is generally described as fairly biodegradable in wastewater treatment works (Joss et al., 2006; Kruglova et al., 2014), in soils (Al-Rajab et al., 2010) and in aqueous environments (Jiskra and Hollender, 2008;

Poirier-Larabie et al., 2016), different environmental conditions can lead to varying degrees of persistence (Baena-Nogueras et al., 2017).

These data strongly suggest that while dilution decreases the concentration of a chemical in the solution, it can increase its persistence. The latter is most likely caused by the concomitant dilution of the bacterial community and the substrate concentration represented by increasing lag phase of acclimation of the bacteria responsible for the contaminant biodegradation (Maier et al., 2009; Swinnen et al., 2004). Such a lag phase is a measure of the resilience of the degrader to such an environmental stress before returning to the conditions necessary to catabolise the contaminant (Ramadan et al., 1990). Furthermore, it has been shown that such a dilution effect might be related to the dilution of other wastewater components such as salts, which indirectly affect the species lag phase duration prior to degradation of the contaminant (Robinson et al., 2001). The reduced removal from dilution of wastewater influent in a wastewater treatment plant has been reported (Joss et al., 2006) and is considered as a factor to be avoided to increase effluent treatment efficiency.

The disappearance of ACT in the abiotic batch was extensive, being 40-50 % within 20 days (Figure26F), but the degradation rate was much slower than in the combined sorption-biodegradation batch (Figure 26E), where removal by biodegradation occurred in a few hours (< 12 h).

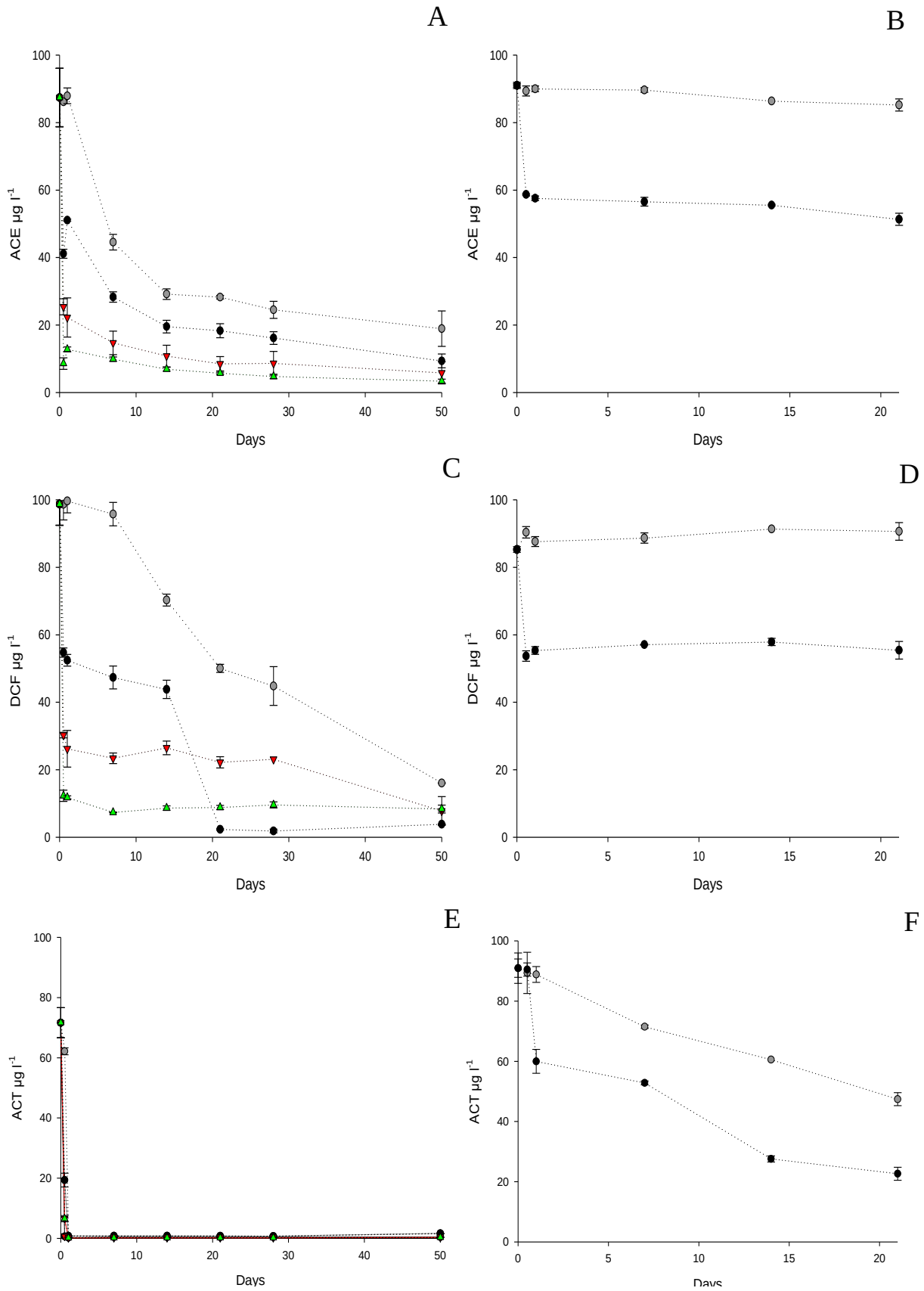

Figure 26 Sorption-biodegradation and the sorption/hydrolysis only respectively of acebutolol (ACE) (A, B); diclofenac (DCF) (C, D); acetaminophen (ACT) (E, F) in untreated wastewater at several dilution factors (DF).

As such, dilution did not visibly influence the biodegradation rate of ACT since the latter was faster than the experimental time designed for batch equilibration. In the study of Lin et al. (2010) consumption of 50 µg L^{-1} was completed with half-life (DT_{50}) of 2.1 days, compared with 3 days for Yamamoto et al. (2009), and 38 days for Baena-Nogueras et al. (2017).

The slower disappearance rate observed in such experiments could be explained by the use of river water, which has a lower dissolved organic matter (DOM) concentration and lower microbial biomass as well as different bacterial communities than untreated wastewater. Such differences highlight the likely variable rates of biodegradation for APIs entering the environment in wastewater as treated effluent or untreated sewage (Tappin et al., 2014, 2012).

CBZ, NVR, VLS, and AMI were persistent over the 50 days of the experiment (Figure 27A, C, E, G), consistent with their known metabolic stability (Nassar et al., 2004; Siddiqui et al., 2011). The only effective natural attenuation processes occurring in the impact zone for these APIs were sorption and dilution.

The neutral species NVR and CBZ have a similar chemical structure which can explain their comparable behaviour (Bagnis et al., 2018b). Their sorption was moderate (10-20 %), consistent with their similar lipophilicity (log K_{ow} 2.7 and 2.5 for NVR and CBZ, respectively); no further losses were observed in either the biotic and abiotic batches (Figure 27A, B). Whilst sorption and dilution were the main factors controlling attenuation of CBZ and NVR in both biotic and abiotic batches. A positive deviation from the expected concentration occurred after dilution suggesting desorption.

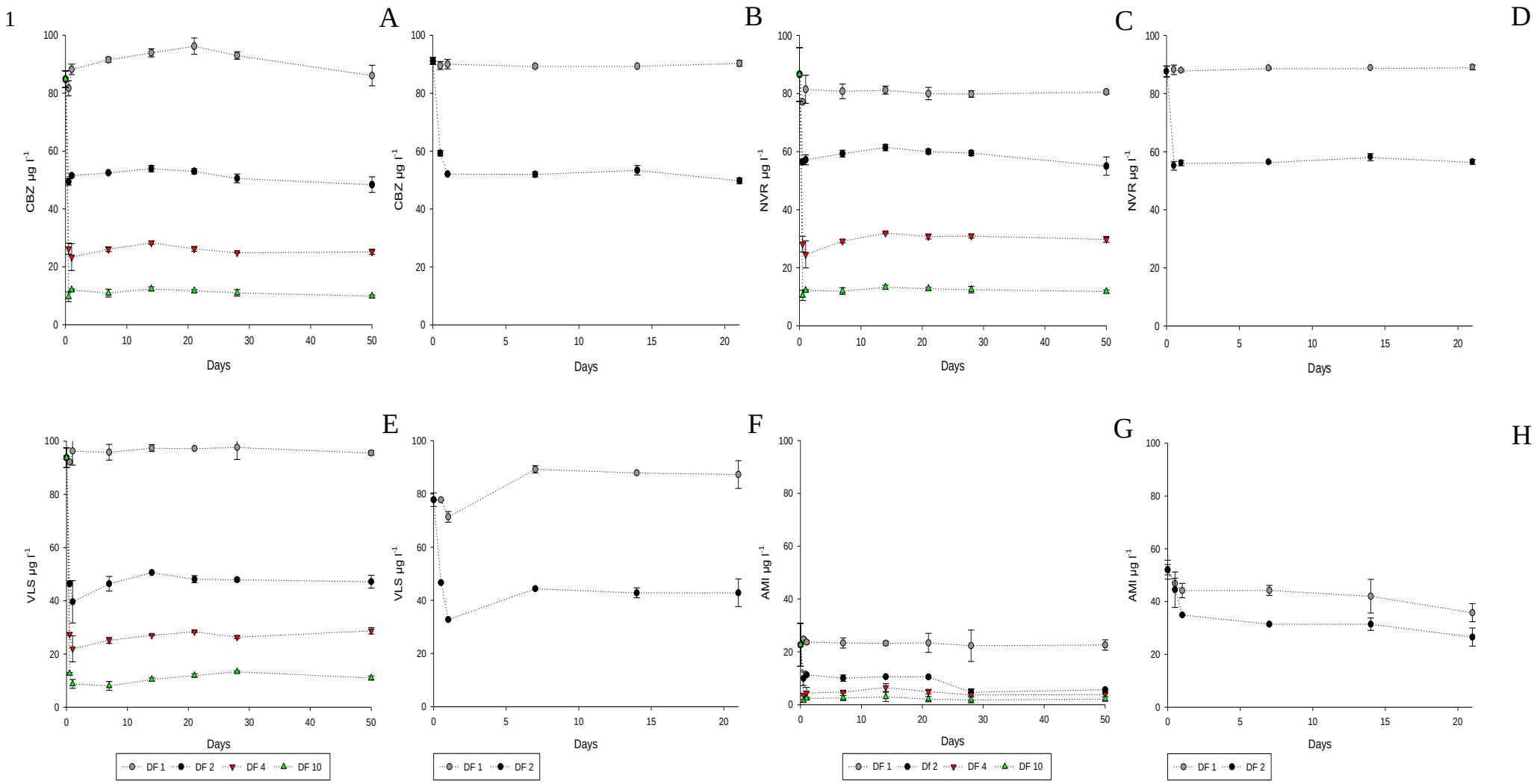

Figure 27 Sorption-biodegradation and the sorption only respectively of carbamazepine (CBZ) (A, B); nevirapine (NVR) (C, D); valsartan (VLS) (E, F); amitriptyline (AMI) (G, H) in untreated wastewater at several dilution factors (DF) 1, 2, 4 and 10.

The high frequency of detection of NVR in the environment (K'oreje et al., 2016; Madikizela et al., 2017; Ngumba et al., 2016) has been attributed to both its widespread use and to poor removal efficiency in wastewater treatment plants (Madikizela et al., 2017). The work of Vankova et al.(2010) provides the only available information on the biodegradability of NVR and the results of this study were consistent with that study, which suggested high recalcitrance in wastewater treatment works. Due to the almost identical behaviour of CBZ, further investigation to evaluate its applicability as a possible marker for sewage contamination (Lim et al., 2017) is recommended, especially targeted to African countries where it is widely consumed (Madikizela et al., 2017).

Little information is available about the natural attenuation of VLS (Bergheim et al., 2014; Mandaric et al., 2019). The distribution of ionisable APIs is controlled by both lipophilicity and charge, expressed by the ionization constant (pKa). In general, the concentration values for the different dilutions, reported for the ionisable compounds used in this study, corresponded to the expected calculated arithmetical value plus desorption, when the latter occurred, in accordance with the compound lipophilicity and speciation (Bagnis et al., 2018a). The results for VLS contrasted with reported data, which described a relatively fast biodegradation with consequent detection of transformation products such as valsartan acid, de-alkylated valsartan and amino-valsartan (Helbling et al., 2010; Kern et al., 2010). These differences highlight the variability in behaviour of this API with the experimental conditions employed. While only a few studies on the persistence of AMI have been documented, these report high persistence in both water (Baena-Nogueras et al., 2017) and agricultural soils (Li et al., 2013), in agreement with the data from this study (Figure 27G).

It is worth noting that the results of this work showed a different degree of sorption between the biotic and the abiotic experiments (Figure 26 and Figure 27). For instance, the anionic VLS showed approximately 20 % of sorption in the abiotic experiment compared with no sorption under biotic conditions (Figure 27E and F). The same behaviour was observed for the negatively charged DCF (Figure 26C and D). In contrast, the positively charged AMI had lower sorption under abiotic conditions (Figure 27G and H). In fact, at DF 1, 80 % the compound was adsorbed in the biotic batch as opposed to 50 % in the abiotic one. Such behaviour has been ascribed to the difference of specific surface area for ionic exchange determined by the presence/absence of a bacterial biofilm on the sorbent surface (Carlson and Silverstein, 1998; Headley et al., 1998; Wunder et al., 2011). The bacterial extracellular polymeric substances are composed of anionic (e.g. $—COO^-$, $—SH^-$, $—SO_4^-$, HPO_4^-), cationic (e.g. $—NH_3^+$), and apolar (e.g. aromatic) functional groups, which at the experimental pH (7.5) are predominantly negatively charged (Wunder et al., 2011). This translates to additional surface sites for exchange of positively charged compounds and the repulsion of negatively charged ones; thus, respectively increasing and decreasing sorption. Such differences between the environmentally more realistic biotic system and the artificial abiotic system highlights a technical bias in the experimental evaluation of the distribution of ionisable chemicals, such as APIs.

5.3.2. APIs persistence through the impact zone

Data from the experiments was used to model the environmental concentrations of APIs within and beyond the impact zone and to determine its extent. The end of the impact zone is defined as the point at which BOD reaches background environmental levels (< 1 – 8 mg L^{-1}) (Schwarzenbach et al., 2003). Therefore, the experimental DOC

was measured and used in the mathematical model (2) derived from the linear correlation of DOC and BOD (R^2=0.86) obtained from a large set of experimental data for untreated wastewater (Kwak et al., 2013). Through this correlation it was possible to estimate the temporal end boundary of the impact zone.

According to the model, BOD decreased most rapidly in the first day (Figure 28), achieving a plateau which constitutes the recalcitrant or slowly degrading organic load present in the mixture (dashed red line in Figure 28) (Dignac et al., 2000). The percentage of refractory DOC in wastewater can be highly variable, but roughly comprised 10-30 % of the initial amount (Dignac et al., 2000; Reynolds, 2002), consistent with the generated data (Figure 28). The recalcitrant DOM could not be excluded from the simple modelling approach adopted in this study and therefore the plateau was considered as the lowest level of observable BOD, and consequently marked the end of the impact zone (A.I.S.E./CESIO, 1995). The end of the impact zone was calculated to be reached in approximately 24 hours irrespective of dilution.

The modelled data suggested that six of the seven APIs investigated in this study would persist through the impact zone and occur at the initial observed concentration beyond its end boundary. Furthermore, although ACT was quickly biodegraded, the high rate of input and patterns of use could lead to pseudo-persistence in the natural environment, leading to the occurrence in concerning concentrations (> 0.01 µg L^{-1}) beyond the impact zone end boundary.

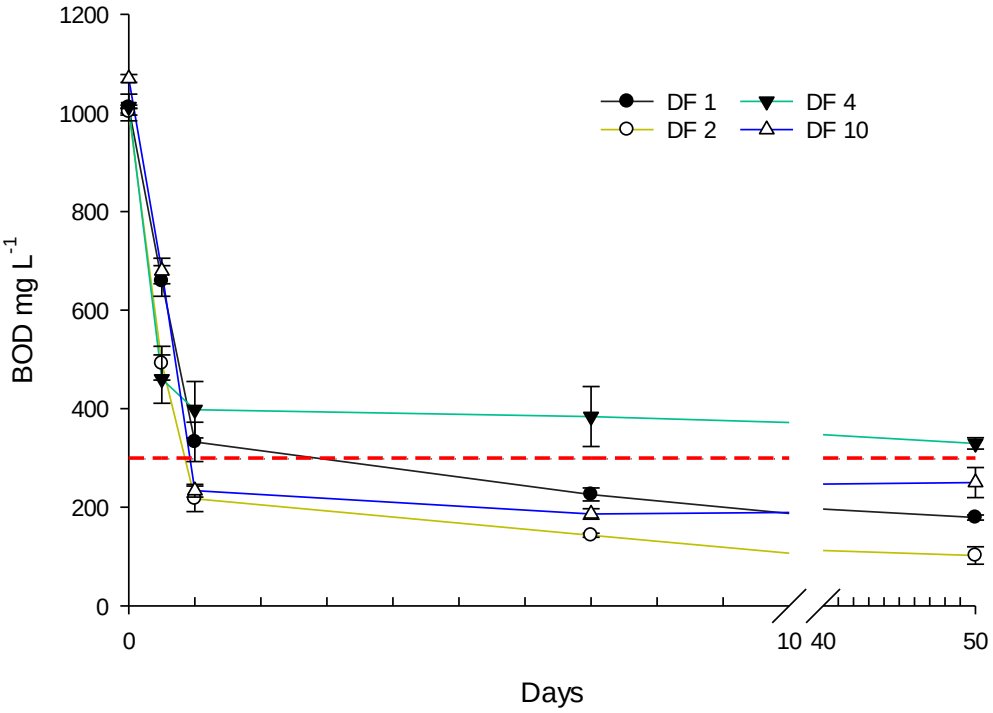

Figure 28 Evolution of the biochemical oxygen demand (BOD) along the experimental time of 50 days for the dilution factors DF 1, 2, 4 and 10; the dashed red line corresponds to the threshold of the 30% of the combination of recalcitrant dissolved organic matter (DOM) and biomass.

Nonetheless, it must be highlighted that uncertainties are likely to be associated with both the model applied for the calculation of BOD and the calculation of the 24h estimate when applied to a real environmental setting. These uncertainties are related to the environmental complexity of each individual scenario which translate in likely varying DOM composition and consequently variable rates of consumption; therefore, further studies are necessary for the development of a more accurate model.

5.3.3. Implications for an environmental risk assessment approach

This work provides novel, robust data that could inform for the future development of an ERA approach for the impact zone with regard to APIs. The data presented here suggests that natural attenuation in the impact zone receiving untreated or poorly-treated wastewaters might not be enough to significantly restrict the environmental burden of APIs within its boundaries. In fact, even at the highest rate of biodegradation, the initial concentration of APIs in the impact zone could be expected beyond its end boundary.

According to this investigation, while low levels of dilution (< 10) reduce the API concentration, the rate of biodegradation also slows increasing the risk of environmental persistence and likely occurrence of the API beyond the impact zone boundary. The dilution level of down-the-drain chemicals in surface waters is highly dependent on local hydrological conditions, such as seasonal runoff variability and so this must also be considered in the evaluation of the risk (Keller et al., 2014). Therefore, further studies are necessary to evaluate this important factor contributing to the environmental exposure of APIs in the impact zone.

Based on the data presented here, it is possible to propose a possible preliminary ERA approach. In phase 1 of the European ERA a predicted environmental concentration (PEC) above 0.01 µg L^{-1} triggers a tier of tests to refine the understanding of the APIs environmental fate (EMA, 2006). Such calculations assume no biodegradation or loss (to sludge or atmosphere) within the wastewater treatment plant. In the case of DDUW the API concentrations would likely exceed such exposure threshold and as a consequence the development of a dedicated phase with the risk assessment is proposed (Figure 29; proposed as phase 2.2). It does not aim to reproduce the current ERA for APIs but is strongly informed by such protocols and compliments it to take account of DDUW. The

new phase of the ERA would be parallel to the current phase 2, renamed in Figure 29 as phase 2.1, for the evaluation of the PEC for APIs beyond the impact zone generated by the DDUW. This phase is divided in two parts, tier A and B, as for phase 2.1. Tier A is based on the modelling methodology used in this study and therefore constrains the impact zone at 24 h. In accordance with the guidelines for the ERA, the experimental studies necessary to evaluate environmental fate should be based on protocols issued by the European Commission, the Organization for Economic Co-operation and Development (OECD) or the International Organization for Standardization (ISO) (EMA, 2006). The PEC beyond the impact zone would therefore be used to evaluate the environmental risk using the PNEC calculated as from the actual protocol (EMA, 2006).

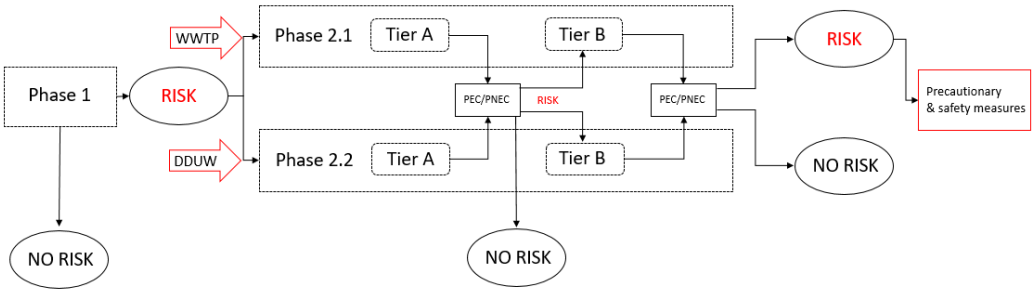

Figure 29 A workflow diagram depicting the protocol to predict environmental concentrations (PEC) in the environmental risk assessment for pharmaceuticals assuming influent treatment in wastewater treatment plant (WWTP) in phase 2.1 (current phase 2), tier A and B, and in the case of direct discharge of untreated wastewater (DDUW), Phase 2.2, tier A and B.

If a risk is still evident further refinement of the PEC would be necessary in tier B, taking into account parent compounds PEC and PNEC as well as the most relevant metabolic fraction (≥ 10 % of amount excreted) (EMA, 2006). In this case, an appropriate model should be developed for the impact zone in the same fashion as SimpleTreat model in EUSES (EMA, 2006).

The approach proposed here would quantify and manage the risk posed by the environmental occurrence of APIs to LLMICs where dilution occurs at factors below 10, such is the case of 53 countries worldwide.

5.4. Conclusions

According to the results of this investigation, the dilution significantly affects the biodegradation rate of DCF and ACE and this might be true for other APIs. ACT instead behaves consistently with previously reported data and it is quickly biodegraded in the wastewater solution regardless the degree of dilution. The other compounds here investigated show high persistence along the experimental timescale. The temporal modelling of the extent of the impact zone allow an estimate of its end at about 24 hours after discharge in the environment, without influence by dilution. The model applied to the APIs natural attenuation data of this study shows a persistence beyond the end of the impact zone. Thus, an ERA approach is proposed, considering the impact zone as a semi-natural wastewater treatment area. The extent of the zone may be modelled with the aim of estimating the APIs concentrations beyond its end, after which the traditional ERA protocol can be applied.

6. CHARACTERIZATION OF THE NAIROBI RIVER CATCHMENT IMPACT ZONE AND OCCURRENCE OF PHARMACEUTICALS: IMPLICATIONS FOR AN IMPACT ZONE INCLUSIVE ENVIRONMENTAL RISK ASSESSMENT

Abstract

The largely uncontrolled release of active pharmaceuticals ingredients (APIs) within untreated wastewater discharged to waterbodies associated with many rapidly urbanising centres is of growing concern owing to potential antimicrobial resistance, endocrine disruption and potential toxicity. A sampling campaign has been undertaken to assess the source, occurrence, magnitude and risk associated with APIs and other chemicals within the Nairobi/Athi river basin, in Kenya, East Africa. The catchment showed an extensive downstream impact zone estimated to extend 75 km, mostly derived from the direct discharge of untreated wastewater from the urban centre of Nairobi city. The exact extent of the downstream boundary of the impact zone was unclear owing to the inputs of untreated wastewater sources from the continuous urbanized areas along the river, which counteracted the natural attenuation caused by dilution and degradation. The most frequently detected APIs and chemicals were caffeine, carbamazepine, trimethoprim, nicotine, and sulfamethoxazole. Paracetamol, caffeine, sulfamethoxazole, and trimethoprim alone contributed 86% of the total amount of APIs analysed and detected along the Nairobi/Athi catchment. The main APIs sources were attributed to the informal settlements and the industrial area in Nairobi City, as well as the Dandora landfill. Also, farming or agricultural sites upstream of the city were likely sources of veterinary APIs. It was shown that there is a potential environmental risk of API ecotoxicological impacts beyond the end of the impact zone, and a medium and high risk exists for fluconazole and sulfamethoxazole respectively.

6.1.Introduction

In the past decade there has been a global increase of production and consumption of APIs in low and low-middle income countries (LLMICs) where the direct discharge of untreated wastewater (DDUW) is prevalent (Kookana et al., 2014). In particular, recent investigations have highlighted the widely-spread occurrence of high concentrations of APIs in pan-African "impact zones", unequivocally ascribed to the poor African wastewater treatment coverage and efficiency (Agunbiade and Moodley, 2014; K' oreje et al., 2016, 2012; Madikizela et al., 2017; Matongo et al., 2015; Ngumba et al., 2016; Schoeman et al., 2015; Wang et al., 2014; Wood et al., 2015). A relatively well studied example of such contaminated areas in Africa is the Nairobi River catchment, flowing through the capital city of Kenya, Nairobi (K'oreje et al., 2012; Mbui et al., 2016; Ngumba et al., 2016). Nairobi was established in the early 1990's with a population of 250,000 and was reputed as a city with high environmental standards, at the point that was named as "the green city in the sun". However, due to rapid urbanization and population growth (3,149,000 officially, but potentially double this in reality) its reputation has changed, and owing to inadequate management of solid wastes and wastewater treatment, the water bodies comprising the Nairobi catchment are severely polluted (Mbui et al., 2016; Mobegi et al., 2016). The wastewater generated in the city's informal settlements and from the centre is mostly directly discharged in the

Nairobi River basin without treatment, leading to a large-scale "impact zone" characterized by the occurrence of high concentrations of APIs (K'oreje et al., 2016, 2012; Ngumba et al., 2016) together with other emerging and traditional organic contaminants (Kithiia, 2007; Kithiia and Ongwenyi, 1997; Mbui et al., 2016; Mobegi et al., 2016; Njuguna, 1979). The water within the catchment is a critical resource, for irrigation, industry, for potable water after treatment and in some cases, a direct source of drinking water for the very poor.

In this study, the Nairobi/Athi catchment was investigated to understand the nature and extent of the "impact zone" generated by the DDUW as follows: (I) The extent of the impact zone was assessed, through the use of analytical techniques traditionally adopted for monitoring the surface water quality and proposed by Bagnis et al., (2019) for modelling purposes; (II) The occurrence and concentration of a large set of APIs and other chemicals in the impact zone and wider catchment has been quantified; (III) A traditional environmental risk assessment was then performed at the furthest sampling point downstream, assumed as the end of the impact zone. The overarching aim was the provision of scientific sound data for the development of an impact zone environmental risk assessment approach.

6.2.Study area and sampling

The sampling area was located in the Nairobi capital province (1,661 m altitude and 696 km^2 of urban area) which is located in the Nairobi/Athi River catchment (Figure 30). The Athi River is the second largest river basin in Kenya, after the Tana River. This river collects its water from the flanks of the Rift valley, the Aberdare ranges and the Ngong hills. After flowing through vast arid areas of Kenya it discharges its waters in the Indian Ocean at the town of Malindi. This river also receives waters from the Nairobi River, its main tributary. The two main tributaries of the Nairobi River are the Mathare and the Ngong River, which drain Nairobi city centre and the surrounding urbanized zones, including informal settlements, industrial areas and agricultural lands (Kithiia, 2007).

The Mathare and Ngong Rivers are the main tributaries of the Nairobi River and all of them flow through Nairobi city (Figure 30). The two tributaries flow through large informal settlements areas (Mathare and Kibera), the latter also through the city's industrial area. The Nairobi and Ngong Rivers flow next to the large 30 solid acres Dandora landfill as well (Muhonja et al., 2018).

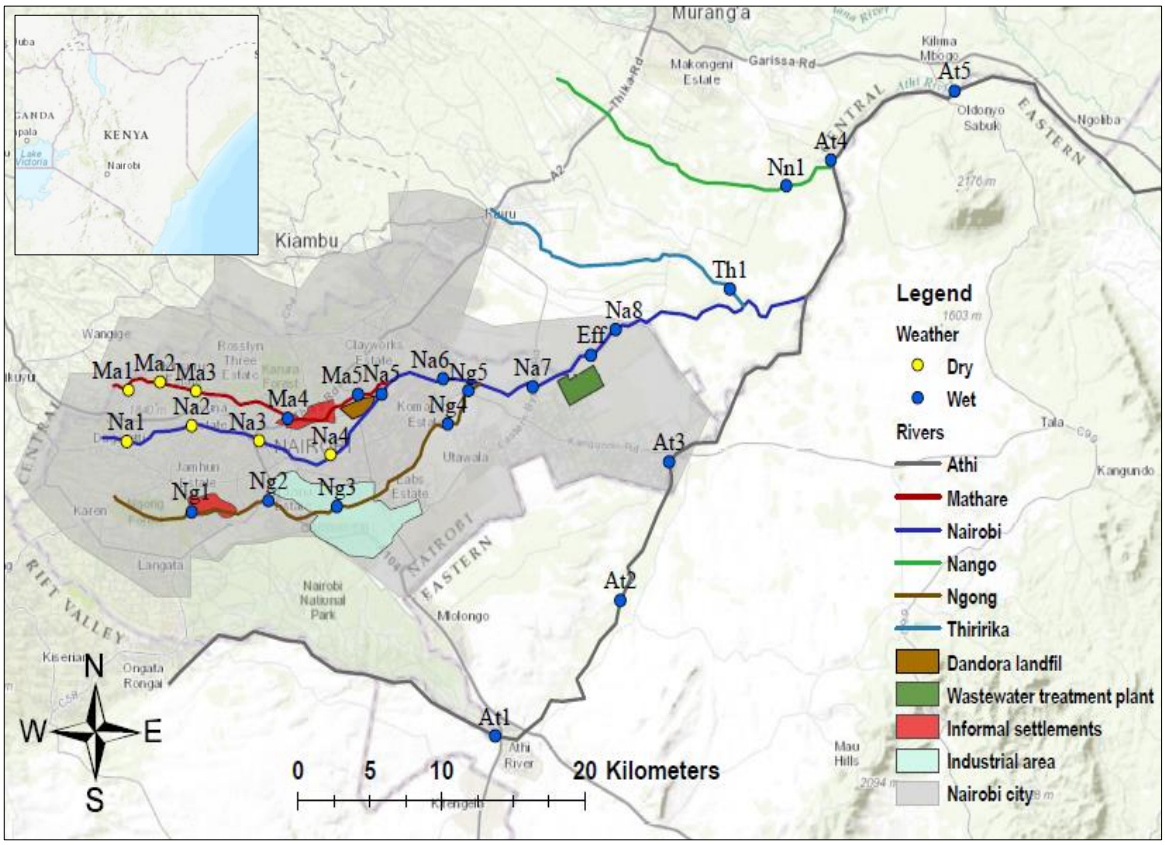

Figure 30 The Nairobi River catchment, the sampling points and the main sites of interest. The water flow is eastwards. "Eff" is the wastewater treatment plant effluent discharge point.

Downstream the city the quality of the Nairobi River water is affected by the effluent from the Dandora wastewater treatment stabilization ponds (WWTP) which treat approximately 27% of the city's population wastewater. Afterwards, the Nairobi River discharges into the Athi River which, after the Fourteen Falls, proceeds to the Indian Ocean. Also, two other minor tributaries of the Athi River were sampled before their confluence, namely Thiririka and Nango Rivers (Figure 30).

Despite the sampling exercise being planned to occur during the dry season, most of the samples (19 out of 26) were collected during the wet season which started

unusually early in mid-March (Figure 30). The sampling campaign comprised a total of 26 sampling points: five along the Mathare River, five along the Ngong River, eight along the Nairobi River, one from the effluent of WWTP, five along the Athi River, and one each from the Thiririka and Nango Rivers at the confluence with the Athi River. The last sample was collected at 75 kilometres downstream of the city, measured from the first upstream sample collected at the Nairobi River.

The source of DDUW was identified as diffuse point sources, such as small pipes from informal settlements, but also significant discharges were occurring from the industrial area (Ngumba et al., 2016).

The samples were collected in 500 ml amber glass bottles and stored on ice and then overnight at 4 °C, and the sample preparation was completed within 24h.

6.3. Calculations

The biochemical oxygen demand (BOD_5) was estimated from the correlation of a data set of TOC and BOD_5 (Comber et al., 2018) and calculated as follows (Kwak et al., 2013):

Equation 11

$$BOD5 = \frac{(TOC + 9.9851)}{0.2876}$$

6.4.Source apportionment of APIs

The Principal Component Analysis (PCA) statistical procedure, validated by Larsen and Baker (2003), was adopted to estimate the source apportionment of the APIs relative to the sampling points along the main stream of the Nairobi/Athi River (Na1 to Na8 and At4 to At5) as representative for the whole catchment, and the effluent from the wastewater treatment plant (Eff) as a source for comparison (Figure 30).

Briefly, with the aim of explaining the variability of the APIs in a minimum number of factors, the data were reduced to Principal Components (PCs) through a factor analysis performed by means of SPSS Statistics 24 (IBM). The analysis was performed setting a Kaiser normalization and a varimax rotation to simplify further the interpretation of the factors.

All the factors originated through the computation are orthogonal to each other reducing the covariance. The first PC corresponds to the component loadings (CL) relative to the linear combination of the original concentration values, and it accounts for the greatest variability. All the other components are in decreasing order of variability. As default, all the components with eigen values less than 1 were excluded. The source emission of each API is indicated by the CL which express the relationship between the PC and the chemical (Dai et al., 2016; Larsen and Baker, 2003).

The most loaded factor scores (> 0.500) for each API were originally considered amongst PCs, with some exceptions in a second analysis comparing the PC with the original concentrations. Thus, the pattern of each PC was critically analysed against literature information to determine the source apportionment.

6.5.Environmental risk assessment

An environmental risk assessment (ERA) was performed using the measured concentrations at the furthest downstream sampling point along the impact zone. The assessment was performed through the risk quotient (2) of the measured environmental concentrations (MEC) of the APIs detected and predicted no effect concentrations (PNEC), retrieved from recent published studies available in the literature.

Equation 12

$$RISK = \frac{MEC}{PNEC}$$

[MEC] and [PNEC] being reported in the same units generating a ratio for the risk. The risk was evaluated based on the guidelines from the European Medicine Agency (EMA, 2006).

6.6. Results and discussion

6.6.1. Impact zone characterization

The rivers physico-chemical parameters at each sampling point are shown in Table 12 (metadata in A3). The distance of each sampling point was measured relative to each river's first upstream sampling point. A concise description of the sampling area and the respective elevation is also provided in Table 12. The mean of the physico-chemical parameters are: pH 8.5, conductivity 570.2 µs cm^{-1}, TDS 245.9 ppm, and temperature 23.7 °C. The temperature varied accordingly to the time of sampling, the lowest in the early morning and increasing along the day until the afternoon (21-29 °C). The altitude difference from the highest point of sample collection (Na1) to the lowest (At5) was of 416m. The BOD_5 was modelled from the TOC concentration (Bagnis et al., 2019). Also, the protein-like dissolved organic matter (PL-DOM) was estimated as a marker of sewage contamination.

The BOD_5 recorded at the sample points along the Nairobi/Athi catchment allowed an estimate of the extent of the impact zone generated by the diffuse DDUW in the Nairobi and Athi River catchments (Table 12). Such estimates were based on the definition of impact zone as the area between the discharge point of untreated wastewater and the downstream point at which the concentration of BOD_5 returns to the expected environmental range (Bagnis et al., 2018a; Bagnis et al., 2019).

The sampling points on the Nairobi and Mathare Rivers upstream of the city centre showed very high BOD_5 of 1136 mg L^{-1} and 1349 mg L^{-1} respectively, and concentrations of PL-DOM of 0.3 mg L^{-1} and 2.0 mg L^{-1} respectively, the latter suggesting a higher contribution from sewage inputs to the Mathare River (Table 12). The range of BOD_5 values recorded at these sampling points were nearly three times above typical values for typical high strength crude sewage (Tchobanoglous et al., 2003), but are in the observed range for industrial effluents (e.g. dyes and pharmaceutical factories) (Lokhande et al., 2011; Pittwell, 1988), which suggest the presence of industrial sources of pollution upstream the Nairobi city centre. However, we do not have evidence of the presence of industrial facilities in the area upstream these sampling points, and additional studies would be necessary to ascertain their presence and nature.

Table 12. A short description of each sampling point area, elevation and the physic-chemical parameters pH, conductivity, total dissolved solids (TDS) and temperature. Also, the biochemical oxygen demand (BOD_5) and the protein-like dissolved organic matter (PL-DOM) are furnished; WWD, wastewater discharge.

Sampling point	River	Area	Distance (km)	Elevation (m)	pH	Conductivity (us cm^{-1})	TDS (PPM)	Temperature (°c)	BOD_5 (mg L^{-1})	PL-DOM (mg L^{-1})
At1	Athi	Downstream Nairobi national park	0	1507	8.1	195	129	25.7	389.4	0.9
At2	Athi	Upstream a tanning plant WWD	13.1	1472	8.8	198	130	28.3	267.8	0.7
At3	Athi	Downstream a tanning plant WWD	23.2	1472	9.6	920	609	26.3	624.2	8.9
At4	Athi	Upstream Fourteen Falls	48.3	1428	8.1	302	199	22	160.3	1.2
At5	Athi	Fourteen Falls	59.3	1392	8	342	224	23.5	292.2	0.9
Eff	WWTP	Effluent WWTP	/	1480	8.8	1099	723	26	520.8	2.8
Ma1	Mathare	Upstream city centre	0	1781	7.4	530	359	21.2	1349.0	2.0
Ma2	Mathare	Dam	2.2	1770	8.3	420	276	24.7	197.4	0.6
Ma3	Mathare	Downstream dam	4.8	1734	7.3	153	101	22.1	901.8	0.6
Ma4	Mathare	Middle of Mathare slum	11.6	1627	8.4	486	320	21.3	297.2	3.7
Ma5	Mathare	Confluence to Nairobi River	19.5	1563	7.8	624	412	20.7	292.7	3.8
Na1	Nairobi	Upstream city centre	0	1808	7.6	362	239	20.1	1136.2	0.3
Na2	Nairobi	Upstream city centre	5.0	1728	8	1050	728	21	490.6	5.4
Na3	Nairobi	City centre	9.9	1680	7.9	768	508	26.2	454.2	4.0
Na4	Nairobi	Between city centre and junction with Mathare River	15.5	1628	7.3	928	616	28.9	638.2	13.2
Na5	Nairobi	Confluence to Mathare River	21.0	1568	8.2	618	409	22.3	292.0	3.4
Na6	Nairobi	Confluence to Ngong River	25.7	1500	8	597	394	23.1	1421.4	2.8
Na7	Nairobi	Upstream WWTP	32.4	1491	8.5	788	522	27	515.2	0.6
Na8	Nairobi	Downstream WWTP	39.38	1459	8.6	935	615	25	503.5	2.9
Ng1	Ngong	Upstream Kibera Slum	0	1714	7.3	94	62.3	19.5	191.9	0.6
Ng2	Ngong	Middle Kibera Slum	5.7	1702	7.6	551	364	22	1115.4	4.5
Ng3	Ngong	Industrial area	11.2	1632	7.8	769	508	25.3	822.3	5.3
Ng4	Ngong	Quarry area	21.2	1547	7.8	817	539	25.2	508.5	3.6
Ng5	Ngong	Confluence to Nairobi River	24.0	1500	8.2	796	526	24	373.5	4.1
Nn1	Nango	Confluence into Athi River	/	1432	7.7	313	205	22.7	249.9	0.3
Th1	Thiririka	Confluence into Nairobi River	/	1430	7.7	171	112	22.5	206.2	0.5

The highest level of PL-DOM (13.2 mg L^{-1}) was observed along the Nairobi River at sampling point Na4 (Table 12), located between the city centre and the confluence to the Mathare River (Figure 30), which highlighted high inputs of sewage contamination from the densely populated city centre. Afterwards, the PL-DOM concentration steadily decreased until sampling point Na8 located after the effluent discharge point from the WWTP, where a slight increase was observed, consistent with the WWTP effluent discharge. The estimated BOD_5 transect along the Nairobi River showed a trend similar to the PL-DOM, with an exception of the sampling point before the confluence with the Ngong River (Na6), where the highest concentration was recorded (1421 mg L^{-1}).

The Mathare River flows through the city's informal settlements and showed an increasing contamination along its length where the concentrations of BOD_5 and PL-DOM were elevated and followed a similar trend (Table 12).

The first upstream sampling point collected along the Ngong River (Ng1) showed a relatively low BOD_5 (192 mg L^{-1}), whilst the highest BOD_5 concentration was recorded just after the informal settlement of Kibera (Ng2) (1115 mg L^{-1}). The BOD_5 concentration steadily decreased until the confluence with the Nairobi River most likely as an effect of dilution (Table 12). The PL-DOM showed an increase to a maximum at Kibera, then kept steady along the length of the river, suggesting continuous input of sewage all along the river which counteracted any dilution or attenuation. Another important contribution to

this impact zone is the extensive industrial area located on the north side of the Ngong River (Figure 30).

The Athi River water quality showed an abrupt increase of PL-DOM at sampling point At3, most likely caused by the contribution of an upstream wastewater discharge point from a tannery. Thereafter, the PL-DOM concentrations gradually started to decrease, and, after the confluence with the Nairobi River, at the last sampling point (At5), the concentration had decreased to 0.9 mg L^{-1}. The BOD_5 within the Athi River were relatively low compared to the rest of the catchment (Table 12). Also, the two smaller influents, Thirika and Nango Rivers, showed both BOD_5 and PL-DOM at the lowest concentrations detected in the catchment, and no influence on the receiving stream water quality.

Overall, a relatively rapid increase of PL-DOM and BOD_5 along the transect upstream and within the urban centre and a rapid decrease thereafter was observed. This was most likely caused by the dilution from rainwater runoff and to an unknown degree, other natural attenuation factors, such as (bio) degradation. The whole sampling area along the Nairobi/Athi catchment was heavily impacted by BOD_5 from numerous industrial and landfill sources as well as diffuse sewage pollution. There is no a clear end of such impact zone as the concentration of BOD_5 at the last downstream sampling point (75km from the first upstream site) was still greater than 8 mg L^{-1}, considered as the threshold for the "severely polluted" categorization of water affected by wastewater pollution (Koncagul

et al., 2017). However, it could be considered an overestimation if there was a significant proportion of recalcitrant DOM in solution, leading to a positive bias in predicted BOD, as suggested by the modelling approach of Bagnis et al., (2018a). If this was the case, then the end of the impact zone could be assumed to be at sampling point At5 for ERA purposes.

6.6.2. Frequency of APIs detection

The samples were collected in 27 locations along the catchment and analysed for the occurrence of 55 APIs belonging to 19 therapeutic categories (Figure 31). Forty-five out of the fifty-five compounds under scrutiny were detected in at least one sampling location, and at least one representative for each of the nineteen therapeutic classes was detected along the entire catchment.

The APIs with the highest frequency of detection (>90%) were caffeine, carbamazepine, and trimethoprim, detected in 100% of the sampling points, and nicotine (81.5%) and sulfamethoxazole (93%) (Figure 31).

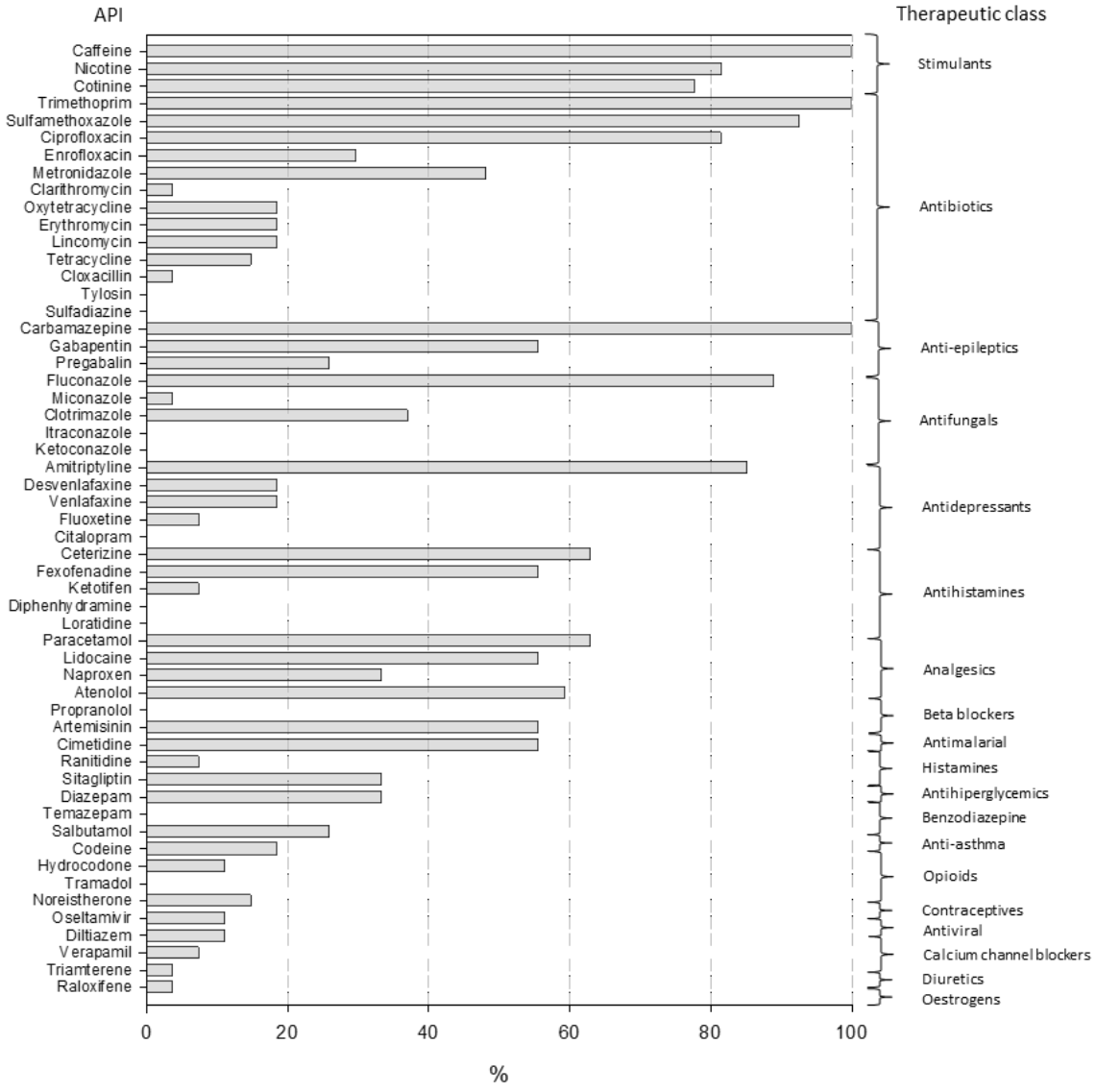

Figure 31 Frequency of detection of the 55 active pharmaceutical ingredients (APIs) at the 27 sampling points (100%) grouped per therapeutic class.

Even though the stimulant caffeine is not strictly an API it was added to the list because of its extensive removal (100%) by conventional wastewater treatment (Sui et al., 2010) that suits it as a marker for DDUW contamination (Dai et al., 2016; Verlicchi et al., 2012). In fact, its detection in all of the sampling points suggested extensive

human-impacted contamination by sewage along the catchment. Also, other human derived stimulants such as nicotine and its main metabolite cotinine were also frequently detected (81.5% and 78 % respectively) supporting the case for untreated wastewater pollution. However, it must also be recognized that the large areas allocated to coffee crops cultivation distributed at the latitudes and altitudes of the Nairobi region, and the presence of tobacco factories in the industrial area of Nairobi, might contribute to the occurrence of these active compounds in surface waters (Barjolle et al., 2017).

In a similar fashion, the antiepileptic drug carbamazepine is also used as a marker for sewage contamination, because of its poor removal and stability, and it was detected at all of the sampling points (100%). This widespread detection is likely due to a combination of sewage contamination and its well-known intrinsic high persistence (Durán-Álvarez et al., 2015; Gasser et al., 2011; Kruglova et al., 2014). In the same therapeutic class were the less frequently detected gabapentin (56%) and pregabalin (26%).

The antibiotics trimethoprim and sulfamethoxazole were detected at high frequency. These compounds were consistently reported as the most frequently detected antibiotics in each United Nations region by aus der Beek et al. in 2016, with the highest maximum concentrations in African countries. Three out of the thirteen antibiotics investigated in this work, namely trimethoprim, sulfamethoxazole, and ciprofloxacin

were detected in a frequency higher than fifty percent; and seven, namely metronidazole, clarithromycin, lincomycin, erythromycin, oxytetracycline, tetracycline, and enrofloxacin in between 10 and 50 % of samples; but cloxacin was detected in less than 10% of the samples collected and tylosin and sulfadiazine not detected at all (Figure 31).

The antifungal fluconazole was detected at a frequency of 89% of the sampling points, followed by clotrimazole (37%) and miconazole (3.7%) belonging to the same therapeutic class. The remaining two antifungals itraconazole and ketoconazole were not detected.

The API amitriptyline was the most frequently detected in the antidepressant therapeutic class (85%), whilst desvenlafaxine and venlafaxine were detected at five sampling points each (19%) and fluoxetine at two locations (7.4%). The antidepressant citalopram was not detected.

Six APIs belonging to the class of antihistamine were investigated. The APIs cetirizine (63%) and fexofenadine (55.6%)were detected at a similar frequency; Ketotifen was detected at only two sampling locations (7%); whilst the antihistamines diphenhydramine and loratidine were not detected at all.

In the class of the analgesic the compound paracetamol was found in seventeen out of the 27 sampling locations (63%) followed by lidocaine (56%) and naproxen (33%). Paracetamol (also known as acetaminophen) has been recognized as the most frequently

detected API globally (Barra Caracciolo et al., 2015), even though it is quickly catabolized by microorganisms and consistently removed from water and wastewater (Baena-Nogueras et al., 2017; Lin et al., 2010; Yamamoto et al., 2009), and therefore absent in samples collected away from any source. Naproxen has a similar environmental behaviour and is quickly eliminated from the aqueous environment (Grenni et al., 2018).

The beta-blocker atenolol was detected at 60% of the sampling points, whilst propranolol was not detected. The antimalarial artemisinin was detected in fifteen sites out of the 27 (56%).

All the other compounds not listed so far fell below the detection frequency of 50%. A total of 11 compounds out of the total 55 were not detected, namely tylosin, sulfadiazine, citalopram, itraconazole, ketoconazole, diphenhydramine, propranolol, miconazole, loratidine, temazepam, and tramadol; 3, namely cloxacillin, triamterene, raloxifene were detected only at one site; 3, namely fluoxetine, ketotifen, verapamil, in only 2 sites; and 3, namely oseltamivir, diltiazem, hydrocodone were detected only at three 3 sampling points. Of these compounds only one, namely fluoxetine, is listed in the Kenyan list of essential medicines (*Kenya Essential Medicines List*, 2016), which might explain their low frequencies of detection.

As an exclusion criterion the compounds not detected or detected in less than 3 sampling points at concentrations <10 ng L^{-1} in the impact zone were excluded from

further statistical analyses as the risk is considered irrelevant in the EU ERA protocol (EMA, 2018) (A4).

6.6.3. Catchment APIs distribution

The Ngong River showed the highest mean and max concentrations of total sum of APIs (Ng2), respectively 31,160 and 55,193 ng L^{-1} (Figure 30 and 31), which were the highest reported along the catchment for the investigated APIs.

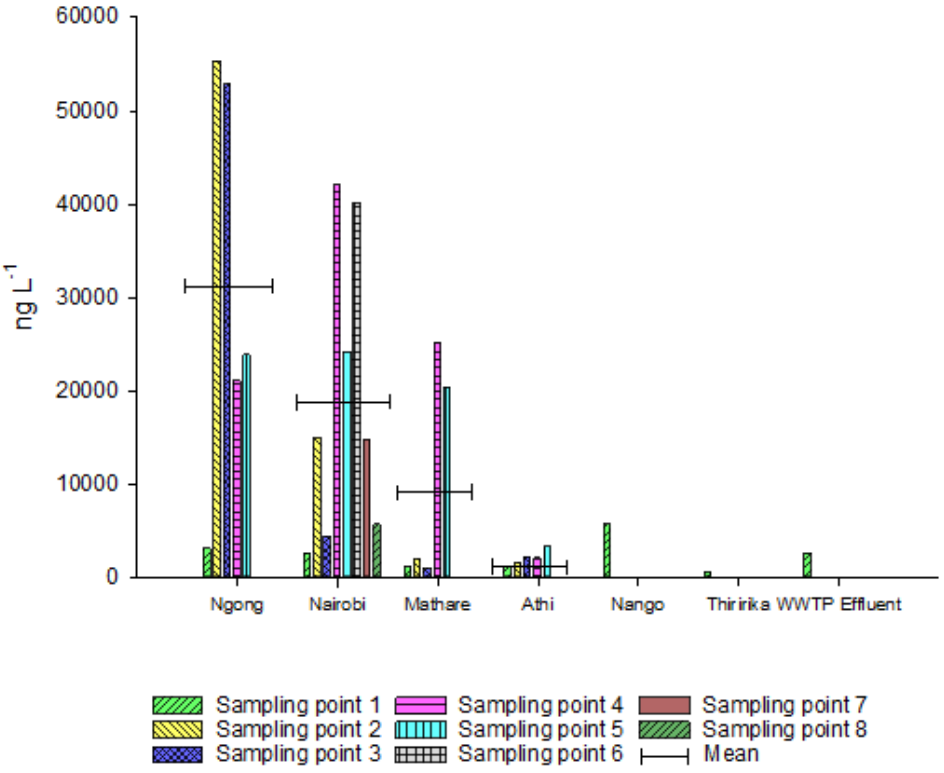

Figure 32 Sum of the 56 APIs at each river sampling point (e.g. Sampling point 1 of Ngong = Ng1; Sampling point 1 of Nairobi = Na1, etc.,) and river mean; the wastewater treatment plant effluent concentration is furnished individually as last right column.

The total concentration of APIs increased from 3052 ng L^{-1} at the sampling point Ng1, upstream the informal settlement of Kibera, to the max total concentration of around 55,000 ng L^{-1} at 5.6 km downstream the slum (Ng2). The subsequent sample was collected in the middle of the industrial area and other informal settlements, Ng3, and showed the second highest total concentration of APIs along this river (52,792 ng L^{-1}). The total concentrations at the last two samples, Ng4 and Ng5, decreased to less than half the maximum concentration, respectively 21,000 and 23,776 ng L^{-1}. Such decrease is very likely due to a combination of reduced input and dilution caused by recent rainfall runoff from the surrounding area. Also, it is likely that (bio) degradation played a role in the decrease of concentrations of the compounds more rapidly catabolised by microorganisms. The pH of the Ngong River increased towards the confluence to the Nairobi River.

The average total APIs concentrations of the Nairobi was 18,560 ng L^{-1} and its maximum concentration was of 41,954 ng L^{-1} recorded at the sampling point Na4 located after the city centre and before the confluence with the Mathare River (Figure 32). The samples collected upstream (Na1, Na2) and in the city centre (Na3) showed a significantly lower concentration with respect to the samples collected downstream (Na4, Na5, Na6). The last two sampling locations showed total concentrations similar to the upstream ones (Na7, Na8), showing a natural recovery of the river water quality with regard to APIs.

The Mathare River showed relatively less API contamination at locations upstream of the city (Ma1, Ma2, Ma3), whilst it was the third highest river for average total amount of APIs (9913 ng L^{-1}) owing to two very polluted sites (25,156 ng L^{-1} at Ma4, and 20,343 ng L^{-1} at Ma5). These last two sampling points are locate in proximity of the Mathare informal settlement and the Dandora landfill (Figure 30) which might explain the sudden increase of APIs.

The Thiririka River was sampled before the confluence with the Nairobi as it could potentially be contaminated by APIs from the upstream urban centre of Githurai located adjacent to Nairobi and therefore contributes to the impact zone. However the results showed a relatively low total concentration of pharmaceuticals (653 ng L^{-1}).

The Athi River showed an average total APIs concentration of 2064 ng L^{-1} and a maximum total APIs concentration of 3255 ng L^{-1}. The first three sampling locations have no influence from the sources of APIs within the city centre. After the sampling point At3 the water quality is influenced by the confluence with the Nairobi River. However, the maximum total APIs concentration was detected at the sampling location At5 (3255 ng L^{-1}), which was likely influenced by the waters coming from the Nango River (5674 ng L^{-1}).

The sample collected from the effluent of the Dandora WWTP showed a concentration much lower than the averages observed in the rivers, which confirms the

importance of the wastewater treatment in reducing the environmental occurrence of APIs (Comber et al., 2018).

6.6.4. APIs individual contribution and occurrence patterns

The analgesic paracetamol was the compound with the highest contribution to the contamination by APIs (47.4%) (Figure 33), and the API occurring in the largest concentration in the Ngong River (maximum 31,003 ng L^{-1}), the Nairobi River (max 24,541 ng L^{-1}), and the Mathare River (maximum 14,180 ng L^{-1}) (Figure 34). These high concentrations contrasted its relatively low frequency of detection (Figure 31) highlighting its well-known rapid biodegradation (Baena-Nogueras et al., 2017; Bagnis et al., 2019; Lin et al., 2010; Yamamoto et al., 2009), which together with dilution, significantly contributed to its decrease of occurrence in the environment and absence at sampling locations far from the source. Paracetamol was detected in other waterbodies of the African continent at concentrations in the same order of magnitude as those recorded in this study (Table 13).

Sulfamethoxazole was the second most abundant API detected in the catchment (15.8%) (Figure 33), occurring as the most abundant API in the Athi River (max 1530 ng L^{-1}, At5), and showed the second maximum concentration in the River Ngong (11250 ng L^{-1}, Ng2) (Figure 34). This compound is used in large amounts globally and widely detected in water compartments, and according to the ERA performed by Straub (2015)

the Nairobi/Athi catchment reported the highest global MEC in a previous study (K'oreje et al., 2012) (Table 13). The well-known wide detection of sulfamethoxazole is owed partly to the highly variable removal rate, caused by the transformation of its metabolites Na-sulfamethoxazole and Glu-sulfamethoxazole back to sulfamethoxazole in WWTP's, which often results in a net negative removal (Göbel et al., 2004). The increase of concentration at the last sampling point with respect to the previous might be caused by a combination of transformation and the contribution of non-identified point sources. Regardless, once in the environment the main mechanism of removal is biodegradation, whilst photodegradation is significant only on a surface shallow layer (Straub, 2016).

The stimulant caffeine showed the third highest contribution (18.1%) and maximum concentration in the River Ngong (10891 ng L^{-1}). This compound was detected in South African water bodies in comparable concentrations (Agunbiade and Moodley, 2016; Matongo et al., 2015) (Table 13).

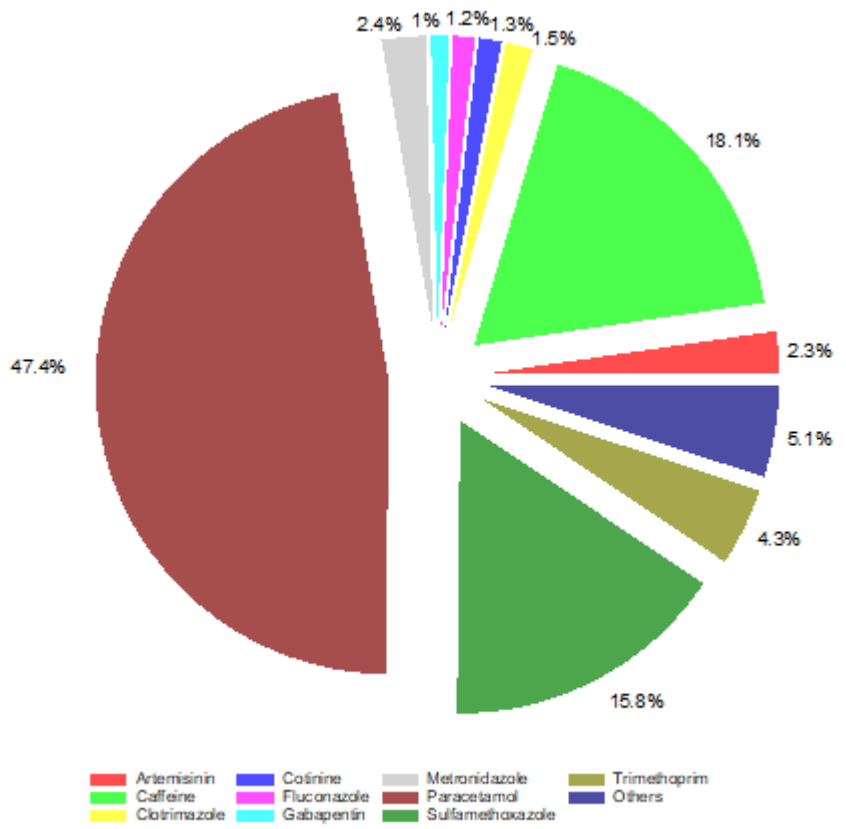

Figure 33 Percentage contribution of each API (sum of each sampling point concentration) to the total concentration of APIs detected along the Nairobi/Athi catchment. The slice "others" contains the compounds occurring for less than 1%, for ease of analyses.

The antibiotic trimethoprim also showed an important environmental load relative to the total APIs (4.3%) and it was detected at all sampling sites. The maximum concentration (3345 ng L^{-1}) was recorded downstream of the Dandora landfill, suggesting a leachate contribution from this potential secondary environmental source of APIs, as previously observed in other studies (Clarke et al., 2015; Masoner et al., 2014). This antibiotic was previously detected by other studies concerning African water bodies, with reported concentrations consistent with this study (Table 13).

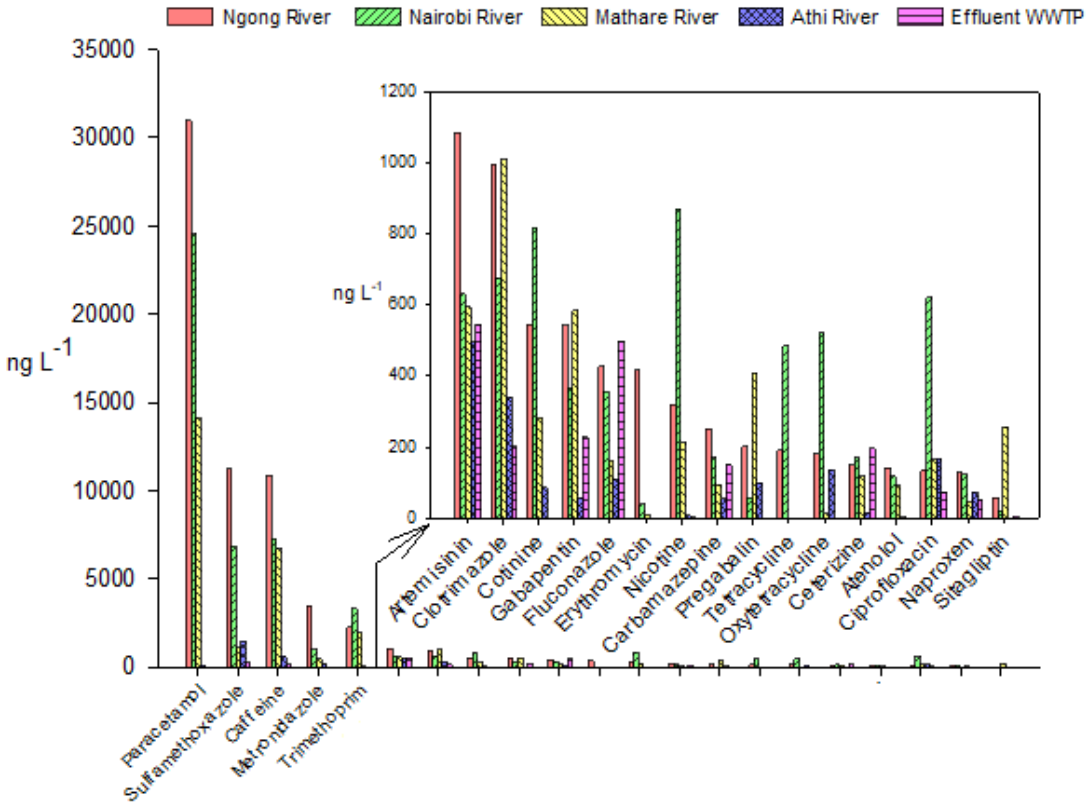

Figure 34 Maximum concentration detected in nanograms per litre of each active pharmaceutical ingredient (API) in each River and wastewater treatment ponds (WWTP) effluent.

These four compounds alone contribute for the 85.6% of the total amount of APIs detected along the Nairobi/Athi catchment. Two of these are antibiotics might strongly contribute to the reported occurrence of antibiotic resistant environmental pools (Subirats et al., 2017).

The other compounds with a contribution higher than 1% were the antibiotic metronidazole (2.4%), the antifungals clotrimazole (1.5%) and fluconazole (1.2%), the stimulant cotinine (1.3%), and the antimalarial artemisinin (2.3%). All the APIs

contributing to less than 1% were grouped in one category that contributes to the 5.1% of the total (Figure 33).

In Table 13 are reported the concentrations of a list of APIs detected both in this study and in other studies on the African continent. For all of them the concentrations of reported APIs are generally similar. Carbamazepine, however, shows concentrations much larger in the study of K' oreje et al., (2016) than this study, both performed in the same catchment. This is probably because of the different sampling periods, in fact the latter study was performed during a dry season, whilst this work was performed during a wet season, which results in a high dilution from rainfall runoff, reducing the concentration of APIs. Also ciprofloxacin was detected in much higher concentrations in the study of Agunbiade and Moodley, (2014), though referring to a different water body in South Africa.

Table 13 APIs detected at the highest concentration (ng L^{-1}) and frequency comparison with previous studies on the African continent (n.a., not available).

API (ng L^{-1})	This work	Ngumba et al. 2016	Matongo et al. 2015	Agunbiade et al. 2014	K'Oreje et al. 2012
Carbamazepine	172	n.a.	n.a.	n.a.	>4000
Caffeine	10890	n.a.	33200	>10000	n.a.
Trimethoprim	3346	2650	290	n.a.	>6000
Nicotine	872	n.a.	n.a.	n.a.	n.a.
Sulfamethoxazole	11250	13765	5320	>8000	>20000
Paracetamol	31003	n.a.	1740	16060	>15000
Amitriptyline	54	n.a.	n.a.	n.a.	n.a.
Ciprofloxacin	168	509	n.a.	>4000	n.a.

6.6.5. APIs source apportionment

The PCA analysis was performed with the purpose to reduce the complexity of the APIs dataset along the Nairobi/Athi River and to allow an easier estimate of the sources of APIs; It resulted in four PCs listed in Table 14.

The first principal component (PC1) showed the highest variance (46%) and was interpreted as the diffuse discharge of untreated wastewater. This is because the PC was highly weighted by APIs and protein-like DOM (PL-DOM) (Table 14). In fact, PC1 was heavily weighted by caffeine, nicotine, and paracetamol which are typically detected in untreated wastewater but completely or highly removed in wastewater treatment plants (Comber et al., 2018; Rosal et al., 2010; Sui et al., 2010). Despite their high recalcitrance to degradation in wastewater treatment plant, carbamazepine, atenolol, and sulfamethoxazole can be also amenable to untreated wastewater discharge as well for the high concentrations (Castiglioni et al., 2006; Comber et al., 2018; Rajab et al., 2013; Rosal et al., 2010). Additionally, in this investigation atenolol is completely absent in the WWTP effluent whilst occurring in river waters, strengthening the direct discharge of untreated wastewater as the main source to the environment (A5).

Table 14 The four principal components (PC1, PC2, PC3, PC4) with the active pharmaceutical ingredients (APIs) and the protein-like DOM (PL-DOM) as variables along the Nairobi/Athi River sampling points. Also, the table includes the estimate source and relative variance.

	PC 1	PC 2	PC 3	PC4
Amitriptyline	**0.876**	-0.090	-0.226	0.000
Artemisinin	-0.347	-0.246	-0.067	-0.495
Atenolol	**0.729**	**0.583**	0.202	-0.121
Caffeine	**0.538**	**0.774**	0.199	-0.079
Carbamazepine	**0.397**	0.160	**0.840**	-0.028
Cetirizine	0.067	0.347	**0.847**	-0.180
Cimetidine	**0.797**	0.320	0.319	0.069
Ciprofloxacin	-0.142	-0.127	-0.136	**0.902**
Clotrimazole	**0.544**	**0.497**	0.435	0.136
Cotinine	**0.956**	0.173	0.098	-0.093
Enrofloxacin	-0.090	-0.249	-0.215	**0.909**
Fluconazole	0.066	0.111	**0.915**	-0.228
Gabapentin	**0.500**	-0.340	**0.574**	-0.367
Metronidazole	-0.098	**0.974**	0.065	-0.074
Naproxen	**0.750**	-0.381	0.436	-0.055
Nicotine	**0.959**	0.082	0.211	-0.010
Paracetamol	**0.735**	**0.553**	0.238	0.051
Sulfamethoxazole	**0.948**	0.116	0.109	-0.176
Tetracycline	-0.140	0.077	-0.143	**0.972**
Trimethoprim	0.003	**0.980**	0.136	-0.02
PL-DOM	**0.876**	-0.157	**0.377**	-0.126
Estimated source	**Untreated wastewater**	**Point sources**	**Wastewater treatment plant effluent**	**Farming upstream city centre**
Variance (%)	46	17	16	9

Despite the information available in the literature about the degradability of the antidepressant amitriptyline is scarce, there is evidence of high persistence (Baena-Nogueras et al., 2017; Bagnis et al., 2019; Li et al., 2013). But, similar to atenolol, its detection in the river waters and the absence in the effluent of the Dandora wastewater treatment plant and the high PC weight (0.88) suggested a contribution of its occurrence from DDUW (S.4). Also clotrimazole, cotinine, naproxen, cimetidine and gabapentin

showed high loading. These compounds were in high concentration in the sampling area between Na4 and Na6 which correspond to the area after the city centre and the suburban area downstream.

The second principal component (PC2) contributes 17% of the total variance. This profile was highly weighted by the APIs atenolol, caffeine, clotrimazole, metronidazole, paracetamol, and trimethoprim. Because of the little significance of PL-DOM to this PC, it was assumed the source of these APIs was linked to poorly defined point sources along the river. The maximum concentration of trimethoprim was recorded at a downstream sampling point (Na6) with respect to the highest concentration of PL-DOM (Na4) (A5). The Na6 sampling area corresponded with the tract of river flowing next to the Dandora Landfill, whose leachate runoff might be deemed as a point source of trimethoprim (Clarke et al., 2015; Masoner et al., 2014). However, also the presence of other sources was considered likely, such as hospitals or veterinary clinics.

The third principal component (PC3) contributed to 16% of the total variance. This PC represented the effluent from the WWTP as it is weighted by only the APIs that were detected in the effluent sample namely carbamazepine, cetirizine, fluconazole, and gabapentin, and moderately weighted by PL-DOM as well, typical of WWTP effluents.

The fourth principal component (PC4) contributes to the 9% of the total variance. This component was highly weighted only by the veterinary antibiotics

ciprofloxacin, tetracycline and enrofloxacin, which were detected at the sampling point Na1 in relatively high concentrations (A5). These APIs represented sources from farming use upstream of the city since they are also used for veterinary purposes (Alexandrino et al., 2017; Granados-chinchilla and Rodríguez, 2017; Peng et al., 2016). However, collecting information about these sources was out of the scope of this work and further investigation could be useful to confirm their origin.

6.6.6. Surface water ERA beyond the end of the impact zone

The data furnished a broad and detailed picture of the extent of the contamination by the direct discharge of untreated wastewater in the Nairobi/Athi catchment and the occurrence of APIs at a point far from the source (At5). The EMA protocol for environmental risk assessment for medicinal active compounds was applied at the last downstream sampling point, assuming that such impact zone acted as a semi-natural wastewater treatment plant as proposed by Bagnis et al., (2019). In ≤ 0.1 insignificant; $\leq$ 1 low; ≤ 10 moderate; > 10 high. The APIs with a MEC <10 ng L-1 (FASS, 2019), and log Kow <4.5 were considered as unlikely to represent a risk (EMA, 2006).

Table 15 are furnished the measured environmental concentrations (MEC) and PNEC of the 14 APIs detected at the sampling point At5, the MEC and PNEC quotient results, and the risk index level as follows: ≤ 0.1 insignificant; ≤ 1 low; ≤ 10 moderate; > 10 high. The APIs with a MEC <10 ng L^{-1} (FASS, 2019), and log K_{ow} <4.5 were considered as unlikely to represent a risk (EMA, 2006).

Table 15 Environmental risk assessment (ERA) calculated from the measured environmental concentrations (MEC) of the APIs detected at the last sampling point At5 (ng L^{-1}) and predicted no effect concentrations (PNEC) available in the literature. The environmental risk index is as follows: ≤ 0.1 insignificant; ≤ 1 low; ≤ 10 moderate; > 10 high. The APIs with a MEC <10 ng L^{-1} (FASS, 2019), and log K_{ow} <4.5 were considered as unlikely to represent a risk (EMA, 2006).

	ERA				
API*	MEC	PNEC	RQ	RISK	REFERENCE
SFX	1529.2	560	2.73	Moderate	AMR Industry Alliance, 2018; Straub, 2016
MTR	182	130	1.4	Insignificant	Bengtsson-Palme and Larsson, 2016
FLC	112.2	250	0.45	Moderate	AMR Industry Alliance, 2018
TRM	64.6	500	0.13	Low	Straub, 2013
CTN	87.5	1000	0.09	Insignificant	Gosset et al. 2017
PAR	45.8	814	0.06	Insignificant	Minguez et al. 2015
AMI	12.8	720	0.02	Insignificant	Minguez et al. 2015
ART	465.6	19000	0.02	Insignificant	Jessing et al. 2009
CFF	634.2	8700000	0	Insignificant	ECHA (https://echa.europa.eu/registration-dossier/-/registered-dossier/10085/6/1)
CBZ	55.2	100000	0	Insignificant	Minguez et al. 2015
GAB	54.6	100000	0	Insignificant	Minguez et al. 2015
FXF	<10	n.a.		Unlikely	
LDC	<10	820000		Unlikely	AstraZeneca, 2017
NCT	<10	100		Unlikely	Oropesa et a. 2017

* AMI, amitriptyline; ART. artemisinin; CFF, caffeine; CRB, carbamazepine; CTN, cotinine; FXF, fexofenadine; FLC, fluconazole; GAB, gabapentin; LDC, lidocaine; MTR, metronidazole; NCT, nicotine; PAR, paracetamol; SFX, sulfamethoxazole; TRM, trimethoprim; n.a., not available.

The PNEC were selected or calculated from the available literature (AMR Industry Alliance, 2018; Bengtsson-Palme and Larsson, 2016; Chen and Ying, 2015; Gosset et al., 2017; Jessing et al., 2009; Minguez et al., 2014; Oropesa et al., 2017; Straub, 2016, 2013). Fexofenadine, nicotine, lidocaine were detected at concentrations below 10 ng L^{-1} and, according to the protocol of ERA for medicines of the EMA (2006), are unlikely to represent a risk for the environment. The log K_{ow} for these APIs is below 4.5,

respectively 2.8, 1.2, 2.3 (Drugbank, 2018h), and therefore there is no need for an additional step involving the assessment of persistence, bio-accumulation and toxicity (EMA, 2006). Fluconaxole presented a quotient of 0.45 which is interpreted as a moderate risk. Sulfamethoxazole also showed a moderate risk as the ratio of MEC and PNEC was above 1 (2.73) but below 10. TRM showed a low risk, whilst the other APIs do not present a significant risk.

Therefore, despite the natural attenuation of APIs occurring along the impact zone it was shown the likelihood of an environmental risk of some APIs ecotoxicological effects beyond its end boundary.

6.7. Conclusions

Based on the data reported above the following conclusions may be drawn regarding the occurrence and potential impacts of APIs within the Nairobi catchment:

- The Nairobi/Athi catchment showed an extensive downstream impact zone mostly derived from the DDUW from the urban centre of Nairobi city.
- The impact zone extended downstream to a distance far from the city. However, its downstream boundary was unclear owing to the inputs of untreated wastewater sources from the continuous urbanized areas along the river, which counteract the natural attenuation caused by dilution and degradation.

- The most frequently detected APIs and chemicals were caffeine, carbamazepine, trimethoprim, nicotine, and sulfamethoxazole.
- Paracetamol, caffeine, sulfamethoxazole, and trimethoprim alone contributed 86% of the total amount of APIs detected along the Nairobi/Athi catchment.
- The main API sources were attributed to the informal settlements and the industrial area in Nairobi City, as well as the Dandora landfill. Also, farming or agricultural sites upstream of the city were likely sources of veterinary APIs.
- It is shown that there is a potential environmental risk of API ecotoxicological impacts beyond the end of the impact zone, and a moderate risk for fluconazole and sulfamethoxazole respectively.

7. CONCLUSIONS

The overall goal of this project was to provide novel and scientifically sound information about the effects of natural attenuation processes on the exposure to active pharmaceutical ingredients in the impact zone generated by the direct discharge of untreated wastewater into surface waters and beyond its boundaries, and to furnish an approach to environmental risk assessment.

The aims outlined in the introduction of the thesis were successfully achieved. The assessment of the effect of low levels of dilution (<10) of the sewage effluent mixed with receiving water on the partitioning of APIs, namely sorption and desorption was described in the relative section and provides sound information on the mechanics driving the distribution of APIs between sorbents and solution, providing some useful endpoints for prediction of environmental concentrations.

Similarly the effect of low levels of dilution (<10) of the sewage effluent mixed with receiving water on the biodegradation rates of selected APIs provided novel insight on the role of the dilution on this natural attenuation process, furnishing a new perspective on an aspect rarely considered in the environmental assessment of chemicals.

The development of a model to determine the extent of the impact zone based on BOD consumption, predicted from DOC concentrations, furnished a very first hint on the approach for developing a model of the impact zone.

The above mentioned laboratory study and novel approaches were validated through a fieldwork real case study which aimed at the characterization of the extent of a real impact zone and the occurrence of APIs in a real environment.

The information generated is of pivotal importance for the development of an environmental risk assessment approach for such areas. In fact, in consideration of the high amount of wastewater generated globally and discharged in the environment with poor or no treatment, it is urgent the need of tools able to predict the risk coming from the exposure to active pharmaceutical ingredients, and to be extended to other classes of chemicals.

As such, from an environmental risk assessment perspective, the impact zone is suggested to be treated as a semi-natural wastewater treatment area and, also, it is proposed the development of a model to allow the prediction of environmental concentrations of APIs beyond the downstream boundaries of the impact zone. In fact, as prove by the data gathered from the Nairobi catchment fieldwork, beyond the impact zone boundaries there could still be a considerable environmental risk. This should be considered even more seriously in the prediction of an increase of use of pharmaceuticals in the developing world.

Therefore, further studies will be necessary to generate laboratory and field data to be used for increasing the understanding of such impacted areas and to allow the

development of tools for the correct evaluation of the environmental risk caused by pharmaceuticals. An important aspect to address in future studies could be the generation of a classification of different impact zone based on hydrological river conditions in order to provide scenarios for modelling, similarly for instance, to traditional models used for the modelling of pesticides environmental concentrations. Another subject requiring additional studies is the role of biofilms in the impact zone on removing APIs, but also as sensitive environmental pool for the propagation of antibiotic resistance genes, a subject already raised to the scientific community attention (Subirats et al., 2017). The use of river water withdrawn from the impact zone for irrigation purposes is important for the transfer of APIs and other organic contaminants in agricultural fields and consequently posing at risk to exposure the consumers in addition to the local ecosystem, another aspect worth further studies.

8. APPENDIXES

A1. Direct aqueous injection-high-performance liquid chromatography/tandem mass spectrometry – list of target APIs

COMPOUND	THERAPEUTIC CLASS
AMITRIPTYLINE	Antidepressant
ARTEMISININ	Antimalarial
ATENOLOL	Beta-blocker
CAFFEINE	Stimulant
CARBAMAZEPINE	Anti-epileptic
CEPHALEXIN	Antibiotic (Cephalosporin)
CETERIZINE	Antihistamine
CIMETIDINE	Histamine H_2 receptor antagonist
CIPROFLOXACIN	Antibiotic (Fluoroquinolone)
CITALOPRAM	Antidepressant
CLARITHROMYCIN	Antibiotic (Macrolide)
CLOTRIMAZOLE	Antifungal
CLOXACILLIN	Antibiotic (Penicillin)
CODEINE	Opioid pain medication
DESVENLAFAXINE	Antidepressant
DIAZEPAM	Benzodiazepine
DILTIAZEM	Calcium channel blocker
DIPHENHYDRAMINE	Antihistamine
ENROFLOXACIN	Antibiotic (Fluoroquinolone)
ERYTHROMYCIN	Antibiotic (Macrolide)
FEXOFENADINE	Antihistamine
FLUOXETINE	Antidepressant

FLUCONAZOLE	Antifungal
GABAPENTIN	Anti-epileptic
HYDROCODONE	Opioid pain medication
ITRACONAZOLE	Antifungal
KETOCONAZOLE	Antifungal
KETOTIFEN	Antihistamine
LIDOCAINE	Analgesic
LINCOMYACIN	Antibiotic
LORATADINE	Antihistamine
METFORMIN	Antihyperglycemic
METRONIDAZOLE	Antibiotic (Nitroimidazole)
MICONAZOLE	Antifungal
NAPROXEN	Analgesic
NICOTINE	Stimulant
NOREISTHERONE	Oral contraceptive
NORFLUOXETINE	Antidepressant/ metabolite of Fluoxetine
OSELTAMIVIR	Antiviral
OXAZEPAM	Benzodiazepine
OXYTETRACYCLINE	Antibiotic (Tetracycline)
PARACETAMOL	Analgesic
PREGABALIN	Anti-epileptic
PROPRANOLOL	Beta-Blocker
RALOXIFENE	Selective estrogen receptor modulator
RANITIDINE	Histamine H_2 receptor antagonist
SALBUTAMOL	Anti-asthma
SERTRALINE	Antidepressant

SITAGLIPTIN	Antihyperglycemic
SULFADIAZINE	Antibiotic (Sulfonamide)
SULFAMETHOXAZOLE	Antibiotic (Sulfonamide)
TEMAZEPAM	Benzodiazepine
TETRACYCLINE	Antibiotic (Tetracycline)
TRAMADOL	Opioid pain medication
TRIAMTERENE	Diuretic
TRICLOSAN	Antifungal
TRIMETHOPRIM	Antibiotic (Sulfonamide)
TYLOSIN	Antibiotic (Macrolide)
VENLAFAXINE	Antidepressant
VERAPAMIL	Calcium channel blocker

A2. Methodology limit of detection (LOD) and Limit of quantification (LOQ)

API	SURFACE WATER	
	LOD	LOQ
	ng/L	ng/L
	n=10	n=10
AMITRIPTYLINE	5.83	11.66
ARTEMISININ	5.75	11.51
ATENOLOL	3.01	6.02
CAFFEINE	11.23	22.46
CARBAMAZEPINE	0.70	1.40
CETERIZINE	10.92	21.84
CIMETIDINE	1.75	3.51
CIPROFLOXACIN	25.83	51.65
CITALOPRAM	0.78	1.56
CLARITHROMYCIN	13.46	26.92
CLOTRIMAZOLE	116.03	232.05
CLOXACILLIN	56.72	113.45
CODEINE	10.32	20.64
COTININE	6.97	13.95
DESVENLAFAXINE	5.33	10.66
DIAZEPAM	0.78	1.56

DILTIAZEM	0.50	1.00
DIPHENHYDRAMINE	8.46	16.92
ENROFLOXACIN	4.23	8.45
ERYTHROMYCIN	1.57	3.13
FEXOFENADINE	4.64	9.29
FLUOXETINE	7.00	13.99
FLUCONAZOLE	5.49	10.98
GABAPENTIN	7.70	15.40
HYDROCODONE	1.65	3.30
ITRACONAZOLE	29.22	58.44
KETOCONAZOLE	2.19	4.38
KETOTIFEN	0.56	1.12
LIDOCAINE	1.33	2.66
LINCOMYACIN	1.27	2.55
LORATADINE	10.31	20.62
METFORMIN	9.21	18.42
METRONIDAZOLE	5.79	11.59
MICONAZOLE	4.73	9.45
NAPROXEN	17.92	35.85
NICOTINE	2.37	4.74
NOREISTHERONE	6.57	13.14
OSELTAMIVIR	4.80	9.61
OXYTETRACYCLINE	8.10	16.20
PARACETAMOL	21.68	43.36
PREGABALIN	10.50	21.00

PROPRANOLOL	7.43	14.86
RALOXIFENE	4.55	9.10
RANITIDINE	2.34	4.68
SALBUTAMOL	5.17	10.34
SITAGLIPTIN	4.17	8.33
SULFADIAZINE	127.97	255.94
SULFAMETHOXAZOLE	1.76	3.52
TEMAZEPAM	17.64	35.27
TETRACYCLINE	12.83	25.65
TRAMADOL	6.69	13.38
TRIAMTERENE	2.39	4.78
TRIMETHOPRIM	0.88	1.75
TYLOSIN	33.89	67.77
VENLAFAXINE	4.92	9.85
VERAPAMIL	1.19	2.37

A3. Metadata of the Nairobi/Athi catchment main streams and influents.

SAMPLING POINT	RIVER	AREA	ELEVATION (M)	COORDINATES	COORDINATE E	pH	CONDUCTIVITY (uS/cm)	TDS (PPM)	TEMPERATURE (°C)	SEASON
NA1	Nairobi	Upstream city centre	1808	S 01 16 34.7	E 036 44 01.7	7.6	362	2.39	20.1	Dry
NA2	Nairobi	Upstream city centre	1728	S 01 16 02.7	E 036 46 28.8	8.0	1050	128	21.0	Dry
MA1	Gathare	Upstream city centre	1781	S 01 14 50.2	E 036 44 06.5	7.4	530	359	21.2	Dry
MA2	Gathare	Dam	1770	S 01 14 33.9	E 036 45 17.2	8.3	420	276	24.7	Dry
MA3	Mathare	Downstream dam	1734	S 01 14 51.9	E 036 46 38.2	7.3	153	101	22.1	Dry
NA3	Nairobi	City centre	1680	S 01 16 33.5	E 036 49 00.6	7.9	768	508	26.2	Dry
NA4	Nairobi	Between city centre and junction with Mathare River	1628	S 01 17 02.2	E 036 51 41.6	7.3	928	616	28.9	Dry
MA4	Mathare	Close to Thika road	1627	S 01 15 47.7	E 036 50 35.2	8.4	486	320	21.3	Wet
MA5	Mathare	Junction to Nairobi River	1563	S 01 14 36.7	E 036 53 47.5	7.8	624	412	20.7	Wet
NA5	Nairobi	Junction to Mathare River	1568	S 01 14 37.9	E 036 53 47.2	8.2	618	409	22.3	Wet
NA6	Nairobi	Junction to Ngong River	1500	S 01 14 38.2	E 036 57 13.3	8.0	597	394	23.1	Wet
NG5	Ngong	Junction to Nairobi River	1500	S 01 14 40.8	E 036 57 12.4	8.2	796	526	24.0	Wet
NG4	Ngong	Quarry area	1547	S 01 15 59.2	E 036 56 06.6	7.8	817	539	25.2	Wet
NG3	Ngong	Industrial area	1632	S 01 18 47.9	E 036 51 56.8	7.8	769	508	25.3	Wet
NG2	Ngong	Middle Kibera Slum	1702	S 01 18 52.7	E 036 47 21.4	7.6	551	364	22.0	Wet
NG1	Ngong	Upstream Kibera Slum	1714	S 01 18 59.0	E 036 46 28.5	7.3	94	62.3	19.5	Wet
NA7	Nairobi	Upstream WWTP	1491	S 01 14 42.8	E 036 59 18.3	8.5	788	522	27.0	Wet
NA9	Nairobi	Downstream WWTP	1459	S 01 13 34.8	E 037 01 34.3	8.6	935	615	25.0	Wet
EFF	WWTP	Effluent WWTP	1480	S 01 13 38.4	E 037 01 29.7	8.8	1099	723	26.0	Wet
TH1	Thiririka	Confluence into Nairobi River	1430	S 01 11 35.5	E 037 06 58.2	7.7	171	112	22.5	Wet
AT4	Athi	Upstream fourteen falls	1428	S 01 06 59.0	E 037 10 31.2	8.1	302	199	22.0	Wet
NN1	Nango	Confluence into Athi River	1432	S 01 07 12.8	E 037 10 32.3	7.7	313	205	22.7	Wet
AT3	Athi	Downstream the tanning plant	1472	S 01 19 55.3	E 037 03 41.2	9.6	920	609	26.3	Wet
AT2	Athi	Upstream the tanning plant	1472	S 01 22 50.0	E 037 02 35.4	8.8	198	130	28.3	Wet
AT1	Athi	Downstream Nairobi national park	1507	S 01 26 37.7	E 036 57 53.7	8.1	195	129	25.7	Wet
AT5	Athi	Fourteen falls	1392	S 01 04 37.7	E 037 15 10.6	8.0	342	224	23.5	Wet

A4. List of APIs excluded from the analysis since not detected or detected in less than 3 sampling points at concentration <10 ng L-1.

API	Criterium	API	Criterium
Citalopram	Not detected	**Loratidine**	Not detected
Cloxacillin	Not detected	**Oseltamivir**	Not detected
Desvenlafaxine	Not detected	**Raloxifene**	Not detected
Diltiazem	<3 sp	**Sulfadiazine**	Not detected
Diphenhydramine	Not detected	**Temazepam**	Not detected
Fluoxetine	<3 sp	**Tramadol**	Not detected
Hydrocodone	<3 sp	**Triamterene**	<3 sp
Itraconazole	Not detected	**Tylosin**	Not detected
Ketoconazole	Not detected	**Venlafaxine**	Not detected
Ketotifen	Not detected	**Verapamil**	Not detected
Lincomycin	Not detected	**Propranolol**	Not detected
Miconazole	1 sp		

A5. List of the concentration of each API used in the principal component analysis at each sampling site and the concentration of the protein-like dissolved organic matter (PL-DOM).

SP\APIs (ng L^{-1})*	*AMI*	*ART*	*ATN*	*CAF*	*CBZ*	*CTR*	*CMT*	*CPR*	*CLT*	*CTN*	*ENR*	*FLC*	*GAB*	*MET*	*NPX*	*NCT*	*PAR*	*SFX*	*TET*	*TRM*	*PL-DOM (mg L^{-1})*
Na1	14	0	0	182	62	0	6	623	0	16	389	37	0	0	0	9	0	183	487	28	394
Na2	35	633	35	1187	81	101	0	224	0	436	115	247	179	0	41	236	7531	3636	0	159	4435
Na3	34	0	40	956	49	62	18	0	0	219	14	162	135	0	0	78	0	2449	0	70	3317
Na4	54	0	119	6433	172	124	86	74	528	821	9	302	369	0	124	872	24541	6890	0	286	10089
Na5	0	474	65	4708	71	172	38	145	0	238	0	155	147	537	0	207	13396	1697	0	2007	3202
Na6	21	0	93	7309	127	162	45	105	678	381	0	358	0	1059	0	233	22770	3093	152	3346	2473
Na7	24	400	79	5824	121	92	0	120	0	214	0	211	193	699	0	104	2645	2305	0	1596	636
Na8	0	0	37	1723	125	143	17	103	0	77	0	338	258	206	0	24	0	1929	0	599	3002
At4	14	498	4	239	42	0	0	131	0	71	0	66	60	0	17	7	0	883	0	40	1052
At5	13	466	0	634	55	0	0	0	0	88	0	112	55	182	0	8	46	1529	0	65	827
EFF WWTP	0	548	0	180	150	197	6	75	202	0	0	499	230	0	54	4	0	317	0	29	2809

*SP: sampling sites; APIs: active pharmaceutical ingredients; AMI: Amitriptyline; ART: Artemisinin; ATN: Atenolol; CAF: Caffeine; CBZ: Carbamazepine; CTR: Ceterizine; CMT: Cimetidine; CPR: Ciprofloxacin; CLT: Clotrimazole; CTN: Cotinine; ENR: Enrofloxacin; FLC: Fluconazole; GAB: Gabapentin; MET: Metronidazole; NPX: Naproxen; NCT: Nicotine; PAR: Paracetamol; SFX: Sulfamethoxazole; TET: Tetracycline; TRM: Trimethoprim; PL-DOM: Protein-like dissolved organic matter

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